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(54) Title: METHODS OF NAD+-DEPENDENT DEACETYLASE INHIBITORS

(57) Abstract: The present invention relates to methods of treating cancer, cardiovascular disorders, and neurological disorders using NAD+-dependent deacetylase inhibitors.

METHODS OF NAD+-DEPENDENT DEACETYLASE INHIBITORS

This application claims priority from Provisional Application No. 60/525,303 filed November 26, 2003, which is incorporated herein by reference in its entirety.

The present invention relates to methods of treating cancer, cardiovascular disorders, and neurological disorders using NAD+-dependent deacetylase inhibitors.

The yeast SIR (silent information regulator) protein complex are responsible to establish, maintain and regulate gene silencing by modifying histones and thus changing chromatin into an transcriptionally inactive state. Among the SIR proteins, SIR2 has recently been identified as a novel type of protein deacetylase. The yeast SIR2 gene is a founding member of a broadly conserved family of deacetyalases found in organisms from bacteria to human. Based on sequence homology, the Sir2 family is categorized as a type III family of histone deacetylases (HDACs), which is insensitive to trichostatin A, a potent inhibitor to both type I and type II HDACs. A unique feature of Sir2/type III HDACs is their dependence on NAD^+ for deacetylation activity. The deacetylation by Sir2 is strictly coupled with the consumption of $NAD+^+$ to produce nicotinamide and a novel metabolite, *O*-acetyl-ADP-ribose. In contrast to type I and type II HDACs which simply remove the acetyl group by hydrolysis to acetate, deacetylation by Sir2/type III HDACs consumes one NAD^+, which takes four ATP to re-synthesize. The Sir2 reaction is also subject to nicotinamide regulation as physiologic level of nicotinamide effectively inhibits the enzyme.

Originally discovered in *Saccharomyces cerevisiae*, the *sir2* mutants cause abnormal expression of regions of the genome that are normally silent. Such regional gene silencing typically results from packaging of transcriptionally active euchromatin into transcriptionally inaccessible heterochromatin. In yeast, *Sir2* is essential for initiating and maintaining silencing at telomeres, mating type loci and ribosomal DNA.

The biochemical mechanism of how Sir2 functioned was only discovered recently. Sequence homology between yeast *Sir2* and *Salmonella typhimurium CobB* provided a clue that Sir2 might be a ribosyltransferase, since *CobB* could rescue defect of *CobT*, a phosphoribosyltransferase gene for vitamin B12 synthesis. This led to a series of meticulous studies that ultimately uncovered a novel biochemical reaction catalyzed by Sir2, the NAD^+-dependent protein deacetylation with nicotinamide and *O*-acetyl-ADP-ribose as by-products.

Sir2 required high level of NAD^+ for deacetylation activity. In a typical in vitro assay with acetylated histone peptides as substrates, the maximal level of deacetylation by Sir2 was only around 30%, even in the presence of 0.5 - 1 mM NAD^+. The concentration of NAD^+ that allowed the reaction to proceed at ~50% of the maximal level was ~100 uM. Presumably, a large amount of NAD^+ was mandatory to sustain Sir2 deacetylation of histones, which were abundant and undergo reversible acetylation and deacetylation cycles on multiple lysine residues in histone.

Nicotinamide, another major input molecule in the NAD^+ salvage pathway, is a feedback inhibitor of Sir2 with an IC_{50} of less than 50 uM. In mammalian tissues, nicotinamide level has been reported between 11 - 400 uM, high enough to suppress

Sir2. Supplementary nicotinamide in growth media also abolishes the Sir2 mediated silencing at rDNA, telomeres and mating-type loci in yeast.

In addition to histones, another major substrate of Sir2 in mammalian cells is p53, the tumor suppressor. p53 is known to be subject to multiple modifications including phosphorylation, acetylation and poly(ADP-ribosyl)ation, which all affect its activity in regulating cell cycle progression and apoptosis. Immunoprecipitation result suggests association of p53 and human Sir2 in nuclei. Antibody specific for acetylated p53 has revealed that Sir2 inhibition by nicotinamide enhances p53 acetylation level in mammalian cells. Maintaining high level p53 acetylation by Sir2 inhibition correlates with high expression of p53 target genes, while Sir2 represses p53-dependent apoptosis in response to DNA damage and oxidative stress It is known that DNA damage induces acetylation and activation of p53 to arrest cell cycle progression or trigger apoptosis.

We have adapted a HDAC assay into a high throughput format for screening Sir2 inhibitors. We have used this NAD+-dependent HDAC assay to search for and identified novel potent small molecule Sir2 inhibitors. These compounds will be useful as a tool to dissect the function of Sir2 and as possible therapeutic agents.

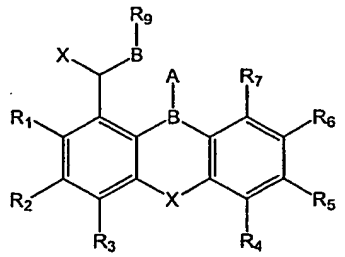
Brief Summary of the Invention

The present invention relates to a method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer, cardiovascular disorders, and neurological disorders

The present invention also relates to a method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase inhibitor to treat cancer.

Detailed Description of the Invention

The present invention involves a method of an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said SIR2 inhibitor can be a compound of formula I:



or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

wherein:

X is O, N, S, P, C=O;

A is a bond, H, CH2, CHR8, -CH2-NH-, -CHR8-NH-, -CH2-NR8-, -(CH2)2-, -CH2-CHR8-;

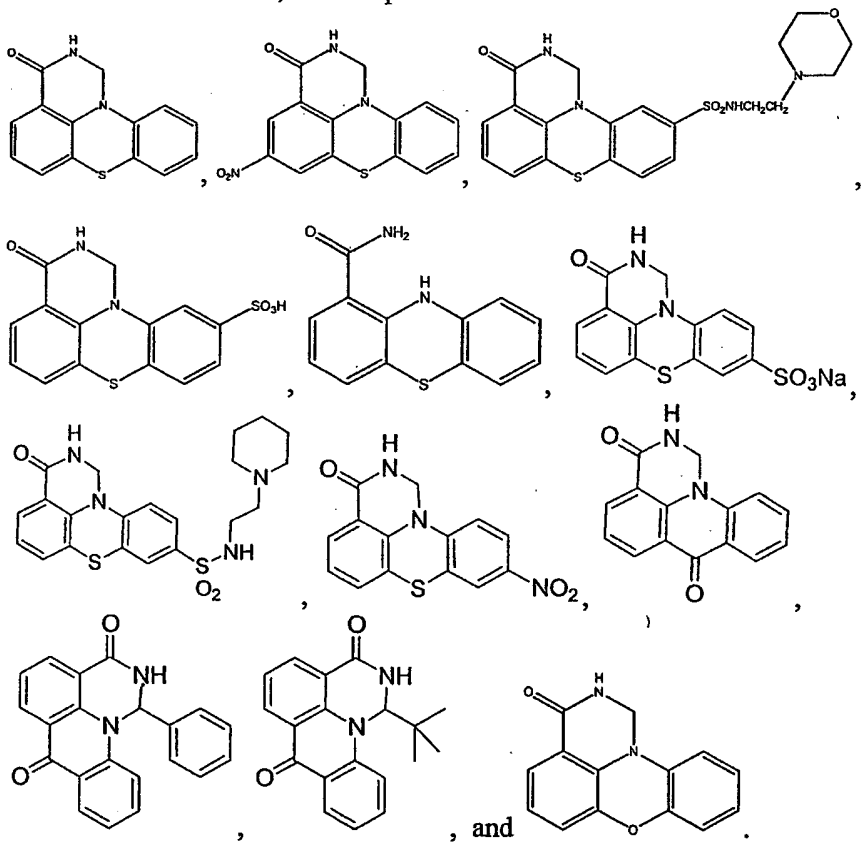
B is C, N, S, C-A, N-A, S-A; and

R1, R2, R3, R4, R5, R6, R7, R8, R9 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or

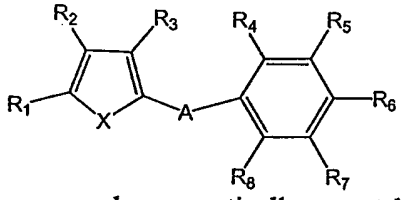
an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

In another embodiment, R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

In another embodiment, the compound can be



The present invention involves a method of an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said SIR2 inhibitor can be a compound of formula II:



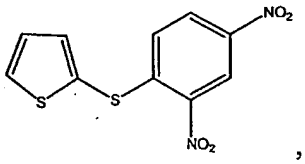
or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

wherein:

X is O, N, S, P;
A is O, S, S=O, SO2, CH2, CHR9, -CH2-NH-, -CHR9-NH-, -CH2-NR9-, -(CH2)2-, -CH2-CHR9-; and
R1, R2, R3, R4, R5, R6, R7, R8, R9 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

In another embodiment, R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

In another embodiment, the compound can be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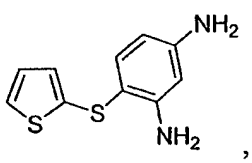
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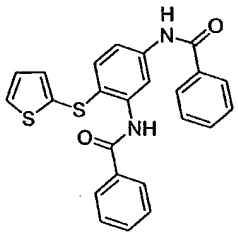
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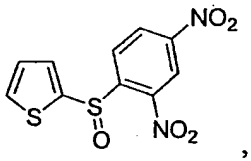
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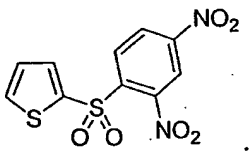
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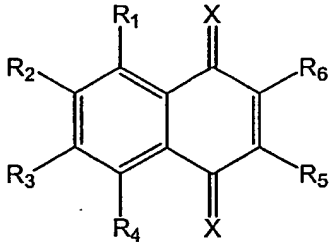
The present invention involves method of an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor can be a compound of formula III:



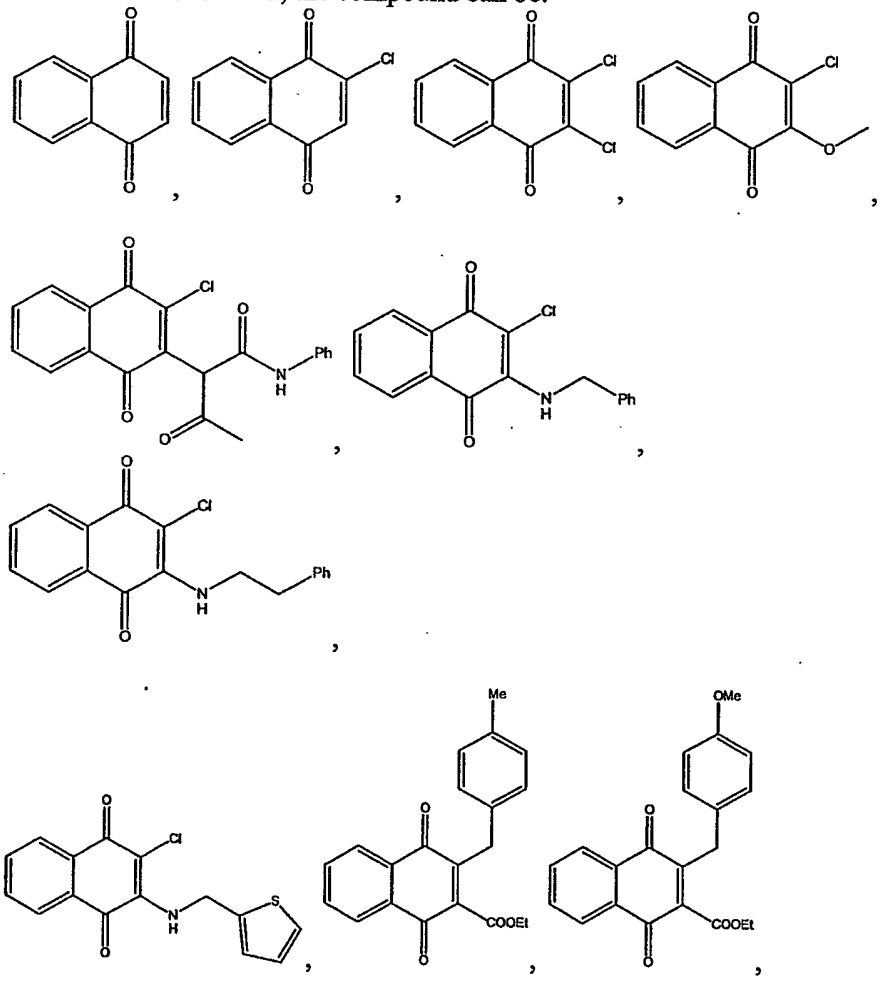
or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

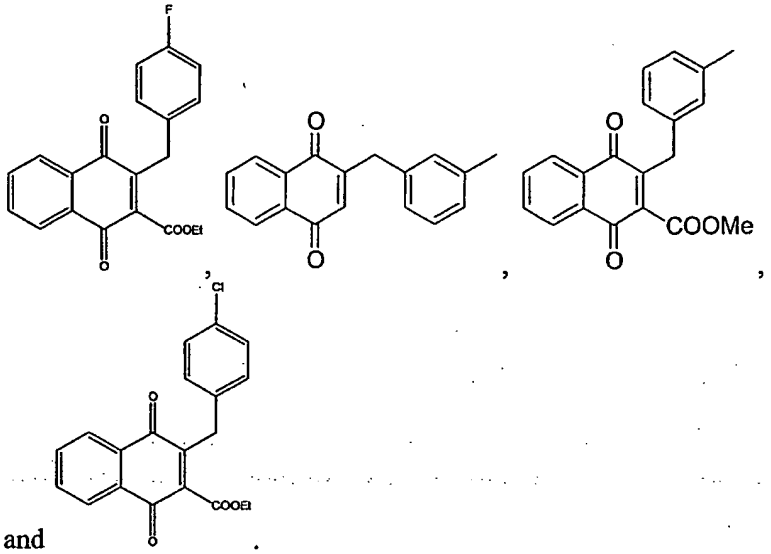
wherein:
X is O, N, S, P; and
R1, R2, R3, R4, R5, R6 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an

optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

In another embodiment, R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 can be H, and -OH.

In another embodiment, the compound can be:





and .

In another embodiment, methods of the present invention treat cancer such as bladder cancer, brain cancer, breast cancer, cervical cancer, head and neck cancer, Hodgkin's lymphoma, lung cancer (small and/or non-small cell), melanoma, non-Hodgkin's lymphoma, ovarian cancer, prostrate cancer, skin cancer, and mixtures thereof.

In another embodiment, a method of an effective amount of a NAD+-dependent deacetylase inhibitor and an anti-cancer agent treat cancer wherein said NAD+-dependent deacetylase inhibitor can be a compound as described herein.

In another embodiment, the anti-cancer agent can be chemotherapy, radiosensitizers, and mixtures thereof.

In another embodiment, a method of an effective amount of a NAD+-dependent deacetylase inhibitor to treat a cardiovascular disorder wherein said NAD+-dependent deacetylase inhibitor can be a compound as described herein.

In another embodiment, methods of the present invention treat cardiovascular disorder such as cardiovascular tissue damage, coronary artery disease, myocardial infarction, angina pectoris, cardiogenic shock, coronary artery bypass surgery, cardiac arrest, cardio-pulmonary resuscitation, and mixtures thereof.

In another embodiment, a method of an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor can be a compound as described herein.

In another embodiment, a method of an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase inhibitor to treat cancer or a cardiovascular disorder wherein said NAD+-dependent deacetylase inhibitor can be a compound described herein.

In another embodiment, the Type I or Type II histone deacetylase inhibitor can be tricostatin A, SAHA, oxamflatin, trapoxin A, FR901228, apicidin, MS-27-275, and mixtures thereof.

In another embodiment, a method of an effective amount of a NAD+-dependent deacetylase inhibitor to treat a neurological disorder wherein said NAD+-dependent deacetylase inhibitor can be a compound as described herein.

In another embodiment, the neurological disorder can be peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, and mixtures thereof.

"Alkyl" refers to a branched or unbranched saturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_1-C_9 alkyl is a straight or branched hydrocarbon chain containing 1 to 9 carbon atoms, and includes but is not limited to substituents such as methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert-butyl, n-pentyl, n-hexyl, and the like, unless otherwise indicated.

"Alkenyl" refers to a branched or unbranched unsaturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_2-C_9 alkenyl is a straight or branched hydrocarbon chain containing 2 to 9 carbon atoms having at least one double bond, and includes but is not limited to substituents such as ethenyl, propenyl, iso-propenyl, butenyl, iso-butenyl, tert-butenyl, n-pentenyl, n-hexenyl, and the like, unless otherwise indicated.

"Alkoxy" refers to the group -OR wherein R is alkyl as herein defined. Preferably, R is a branched or unbranched saturated hydrocarbon chain containing 1 to 9 carbon atoms.

"Carbocycle" refers to a hydrocarbon, cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged. Examples include cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclopentene, cyclohexene, cycloheptene, cyclooctene, benzyl, naphthene, anthracene, phenanthracene, biphenyl and pyrene.

"Aryl" refers to an aromatic, hydrocarbon cyclic moiety having one or more closed ring(s). Examples include, without limitation, phenyl, naphthyl, anthracenyl, phenanthracenyl, biphenyl and pyrenyl.

"Heterocycle" refers to a cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged, with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrolidine, pyrrole, thiazole, thiophene, piperidine, pyridine, isoxazolidine and isoxazole.

"Heteroaryl" refers to an aromatic, cyclic moiety having one or more closed ring(s) with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrole, thiophene, pyridine and isoxazole.

"Derivative" refers to a substance produced from another substance either directly or by modification or partial substitution.

"Effective amount" refers to the amount required to produce the desired effect.

"Therapeutically effective amount" refers to the amount required to treat glaucoma in an animal or a mammal.

"Pharmaceutically acceptable carrier" refers to any carrier, diluent, excipient, wetting agent, buffering agent, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbent, preservative, surfactant, colorant, flavorant, or sweetener, preferably non-toxic, that would be suitable for use in a pharmaceutical composition.

"Pharmaceutically acceptable equivalent" includes, without limitation, pharmaceutically acceptable salts, hydrates, metabolites, prodrugs, and isosteres. Many pharmaceutically acceptable equivalents are expected to have the same or similar *in vitro* or *in vivo* activity as the inventive compounds.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds that possesses the desired pharmacological activity and that is neither biologically nor otherwise undesirable. The salt can be formed with acids that include without limitation acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycero-phosphate, hemisulfate, heptanoate, hexanoate, hydrochloride hydrobromide, hydroiodide, 2-hydroxyethane-sulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

"Prodrug" refers to a derivative of the inventive compounds that undergoes biotransformation, such as metabolism, before exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the inventive compounds using methods known in the art, such as those described by *Burger's Medicinal Chemistry and Drug Chemistry*, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995).

"Treating" refers to:

(i) preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and/or

(iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

"Histone deacetylase" and "HDAC" are intended to refer to any one of a family of enzymes that remove acetyl groups from the epsilon-amino groups of lysine residues at the N-terminus of a histone. Unless otherwise indicated by context, the term "histone" is meant to refer to any histone protein, including H1, H2A, H2B, H3, H4, and H5, from any species. Other histone deacetylases include class I and class II enzymes such as the histone deacetylase is a human HDAC, including, but not limited to, HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, and HDAC-8.

"Histone deacetylase inhibitor" or "inhibitor of histone deacetylase" is used to identify a compound having a structure as defined herein, which is capable of interacting with a histone deacetylase and inhibiting its enzymatic activity. Inhibiting histone deacetylase enzymatic activity means reducing the ability of a histone deacetylase to remove an acetyl group from a histone.

"NAD+-dependent deacetylase" refers to a protein that removes the acetyl groups from a lysine residue of another protein, wherein the deacetylation is coupled to NAD+ (nicotinamide adenosine dinucleotide) cleavage.

"p53-dependent apoptosis" refers to the genetically determined death of a cell that is dependent on, or stimulated by, the p53 gene, a gene that typically inhibits non-natural cell growth, such as that observed in tumors.

"Silence", "silencing" and "silenced" refers to a mechanism by which gene expression in particular regions of the genome are expressed.

"Cancer" or "neoplasms" include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. The term "cancer" is interpreted broadly. The compounds of the present invention can be "anti-cancer agents", which term also encompasses "anti-tumor cell growth agents" and "anti-neoplastic agents". For example, the methods of the invention are useful for treating cancers and radiosensitizing tumor cells in cancers such as ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovary (germ cell) cancer, prostate cancer, pancreatic cancer, penile cancer, retinoblastoma, skin cancer, soft-tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva and Wilm's tumor.

"Carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

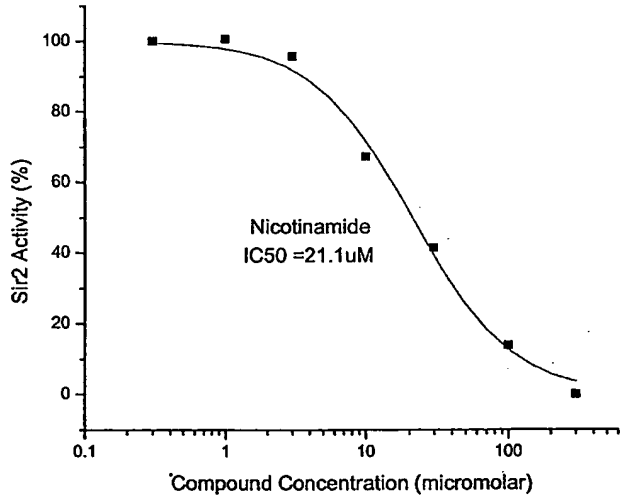
"Sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

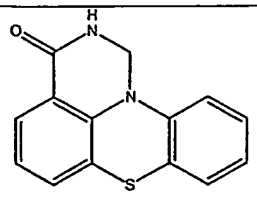
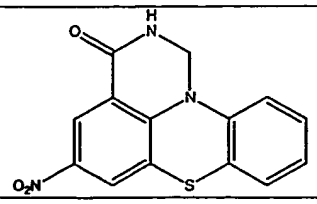
Sir2 Assay

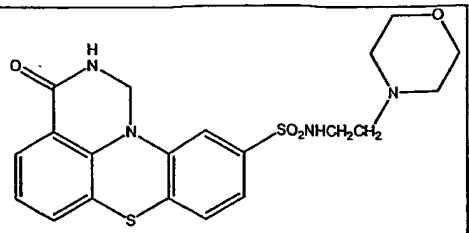
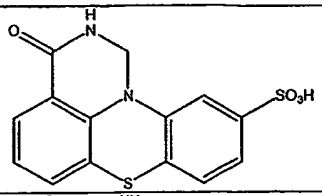
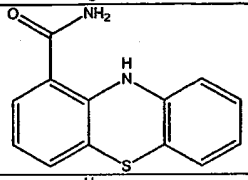
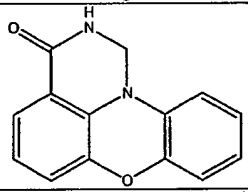
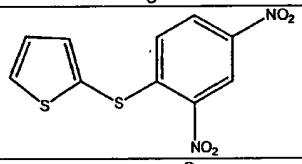
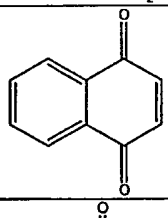
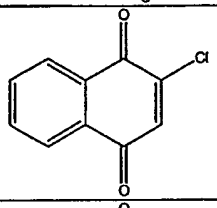
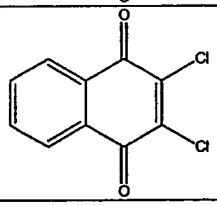
A cDNA encoding the human Sirturin2 was amplified by polymerase chain reaction from human spleen cDNA (Clontech, Palo Alto, California) using a pair of primers with the sequences of 5'- GCGAATTCTCACTGGGGTTTCTCCCTCTC-3' and 5'- GCGGATCCGCAGAGCAGACCGACTCAGATTC -3'. These primers contained the restriction enzyme sites for Bam H1 and Eco RI. The PCR amplified Sirturin2 DNA was digested with Eco RI and Bam H1, and then ligated to the same restriction sites in the pET28a expression vector (Novagen) to create pET28a-Sirturin2, using standard molecular biology procedures. The pET28a-Sirturin2 was transformed into *E. coli* strain Rosetta (LysS) (Novagen) for expression of the recombinant protein with a penta-His tag at the amino terminus. We followed the

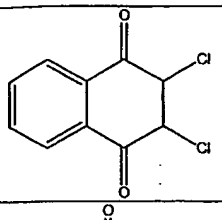
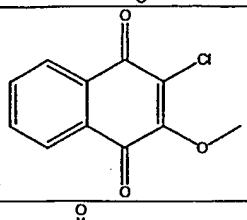
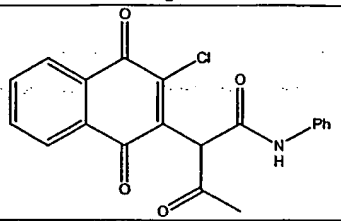
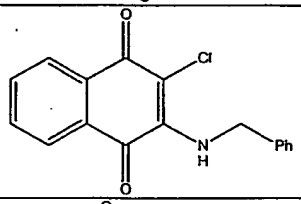
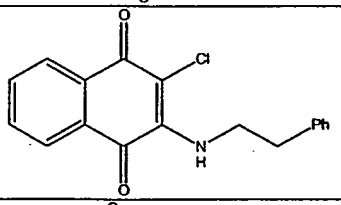
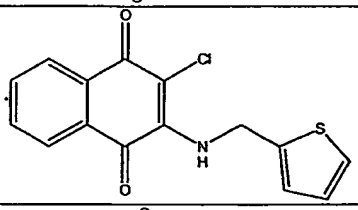
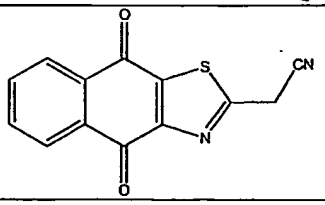
standard procedures for expression and purification of recombinant protein by using nickel His-binding columns according to the manufacturer (Pierce, Illinois).

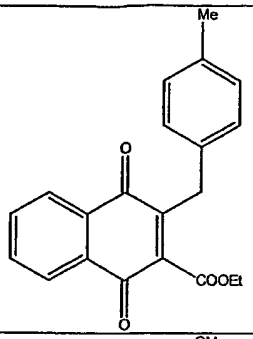
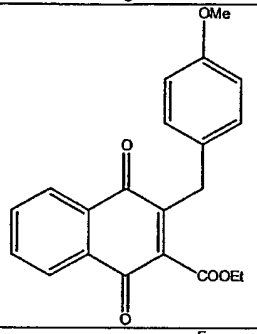
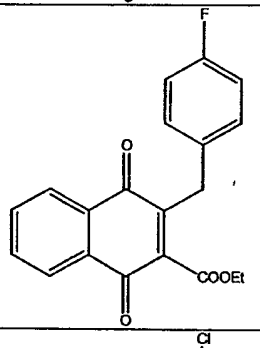
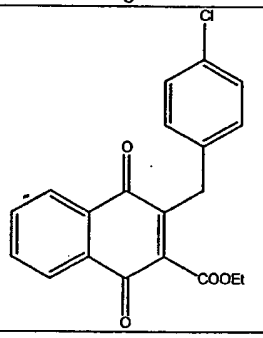
The Sir2 assay is carried out in a 50 µL volume. It consists of 8.75 mM TrisHCl (pH 8.0), 48 mM NaCl, 0.95 mM KCl, 0.35 mM $MgCl_2$, 0.500 mM NAD+, 0.5 mM of Fluor de Lys™ substrate (Biomol), and 5 ng of purified recombinant Sirturin2 enzyme. For determination of the IC_{50}, a typical experiment consists of compound doses at 0.3, 1, 3, 10, 30, 100, and 300 µM. Each dose is tested in duplicate. The stock solutions of compounds tested are prepared in 100% (v/v) DMSO, resulting in a final concentration of 5% DMSO in the reaction. The Sirturin2 enzyme is added last to initiate the reaction. The reaction is carried out at 37°C for 45 minutes and then terminated by adding 50 µL of Fluor de Lys™ deleveloper (Biomol) with 1 mM nicotinamide. Following a 5-10 minute incubation at room temperature, sample fluorescence is read with a fluorimeter (Spectra MAX Gemini XS, Molecular Devices) using an excitation wavelength of 365 nm and an emission wavelength of 450 nm. A typical dose response curve is illustrated in Figure 1 using nicotinamide as an example.



Compound	Structure	IC_{50} (uM)	Inhibition (at 50 uM)	Inhibition (at 100 uM)
1		22.5		
2		20.5		

3		105		
4				14 %
5				43 %
6				76 %
7		3		
8			47 %	
9			70 %	
10		50		

11		34		
12			54 %	
13			91 %	
14			47%	
15			70 %	
16			45 %	
17			65 %	

18		3.6		
19		10.5		
20		15.7		
21		21.7		

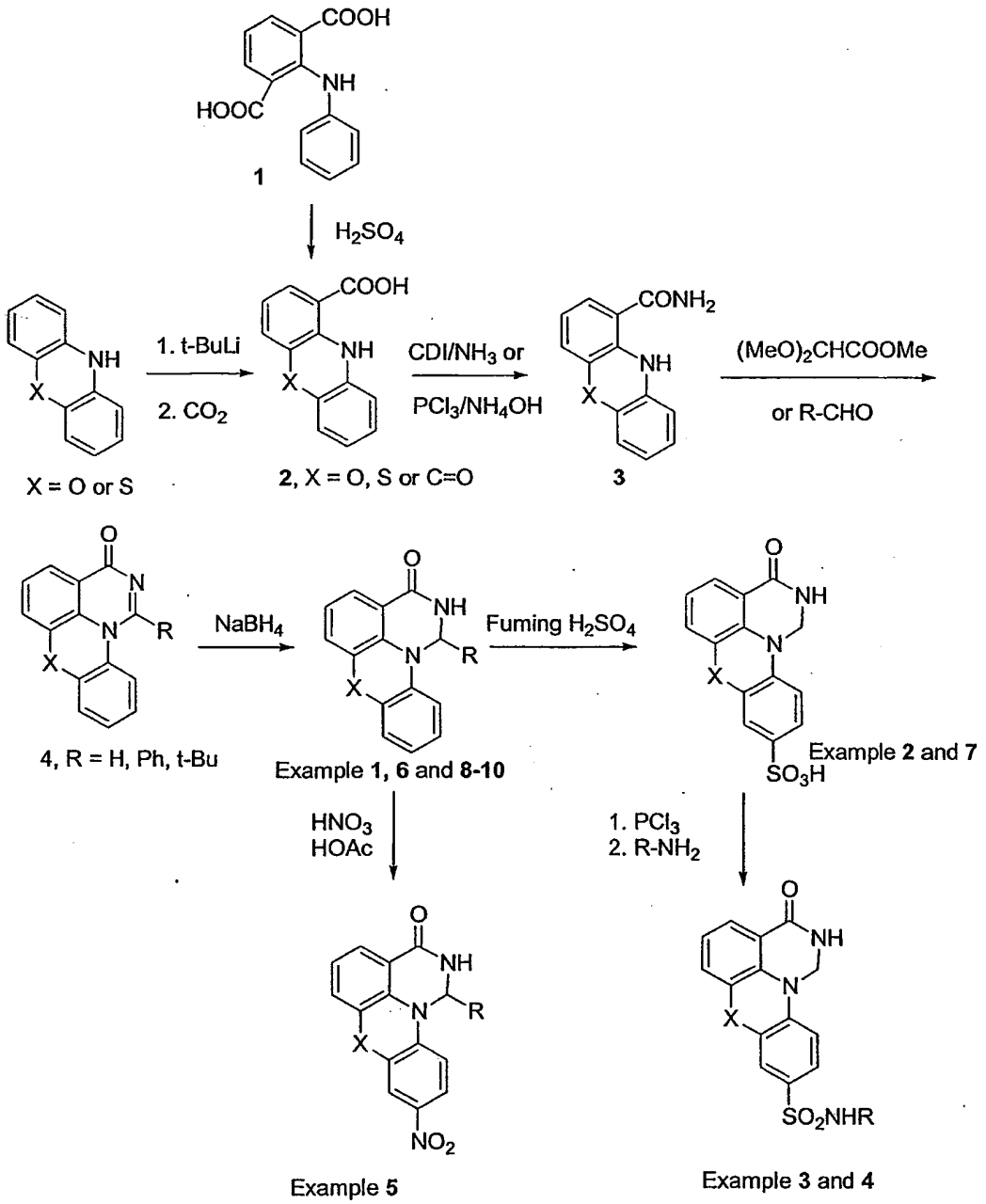
EXAMPLES

1,2-Dihydro-7-thia-2,11b-diaza-benzo[*de*]anthracen-3-one and 1,2-dihydro-7-oxa-2,11b-diaza-benzo[*de*]anthracen-3-one derivatives of this invention are represented by previously defined formula I. As an example, these derivatives can be prepared in a conventional manner as illustrated below by Scheme 1. The tetracyclics,

such as the compounds exemplified bellow, may be generically substituted as set forth in formula I.

Phenoxazine, phenothiazine and phenoxazinone derivatives of Example **1-10** can be prepared fromphenoxazine, phenothiazine or anthrannilic acid as illustrated in Scheme 1. 1-Phenoxazine carboxylic acid or 1-phenothiazine carboxylic acid can be prepared by lithiation at the 1-position of phenoxazine and phenothiazine, followed by quenching with carbon dioxide. For example, 1-phenoxazine carboxylic acid was prepared as follows. To a solution of phenoxazine (5 g, 27 mmol) in anhydrous ethyl ether under N_2 was added t-butyllithium (1.7 M in pentane,16 mL, 27 mmol) at –78 °C. The mixture was stirred until color turned yellow and precipitate formed, and then warmed to room temperature over a period of 20 min. The mixture was cooled to –78 °C again. Carbon dioxide was bubbled in for 10 min. The color of the mixture turned green. The mixture was warmed to room temperature, stirred overnight, and poured into ice/water. The solution was adjusted to pH 10 by adding saturated $NaHCO_3$. The solution was washed with ethyl acetate. The aqueous solution was acidified to pH 2 with 1N HCl. Precipitate was filtered and washed with water to give yellow solid (64.5%). Compound **2** (X = CO) of 9-oxo-9,10-dihydro-acridine-4-carboxylic acid was prepared by intramolecular Friedel-Crafts cycloaddition. A solution of N-(2-carboxyphenyl)anthrannilic acid **1** (16.2 g) and concentrated H_2SO_4 (100 mL) was heated at 100 °C for 4h. The mixture was cooled and poured into ice-cold water.

Scheme 1



The solid was collected and washed with water. The solid was dissolved in dilute aqueous NaOH and filtered. The solution was diluted with the same volume of EtOH and then acidified with glacial acetic acid. The precipitated white product was collected and washed with 50% EtOH to give **2** (X = O, 12.3 g, 81%), mp 340 – 342

°C. LRMS: (M+1): 240; ^{1}H NMR (400 MHz, DMSO-d_6) of **2**: δ 13.75 (s, br, 1H), 11.97 (s, 1H), 8.54 (dd, 1H), 8.45 (dd, 1H), 8.25 (d, 1H), 7.80 – 7.74 (m, 2H), 7.39 – 7.32 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) of **2**: 176.85, 169.46, 141.52, 140.25, 137.22, 134.42, 132.72, 126.21, 122.63, 121.95, 120.93, 120.55, 118.94, 115.35. Several ways to convert carboxylic acid to carboxamide can be used by those skilled in the art. For example, a mixture of the carboxylic acid **2** (X = CO, 1.0 g) and 1,1'-carbonyldiimidazole (1.1 g) in DMF (10 mL) were stirred until it became homogeneous. The mixture was cooled to 10 °C, and a saturated solution of NH_3 in $CHCl_3$ (6 mL) was added. After 1h at room temperature, the precipitate was filtered and washed with Et_2O to give the carboxamide **3** as a yellow solid (0.69 g, 69%). The lactam ring formation from the amide **3** can be carried out by heating the amide with a single carbon source reagents, such as trimethoxy methane and triethoxymethyl acetate in co-solvent of ether/toluene or DMF. For example, a mixture of amide **3** (X =CO, 0.40 g) and diethoxymethyl acetate (50 mL) was heated at 120 °C for 6h. Cooled and the solvents were evaporated in vacuo. The residue was washed with EtOAc to give 2,11b-diaza-benzo[*de*]anthracene-3,7-dione **4** (X =CO) as a yellow solid (0.27 g, 64%), mp 300 °C dec. LRMS: (M-1): 247; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.87 (s, 1H), 8.70 (dd, 1H), 8.61 - 8.58 (m, 2H), 8.41 (dd, 1H), 8.02 - 7.98 (m, 1H), 7.90 (t, 1H), 7.68 (t, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): d 177.00, 167.36, 149.57, 138.04, 137.40, 135.73, 134.55, 132.82, 127.67, 127.38, 127.10, 123.14, 121.94, 119.17, 116.54. Reduction of the N=C double bound of compound **4** desired tetracyclic products. Substituted derivatives of the reductive compounds **4**, such as Example **1** – **10**, can be prepared using common organic methods.

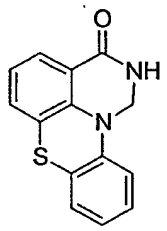
Nitration of compound Example **1** provides 3-oxo-2,3-dihydro-1H-7-thia-2,11b-diaza-benzo[*de*]anthracene-9-sulfonic acid (example **5**). Several nitration methods can also be used by those skilled in the art, include fuming nitric acid or sodium nitrate. Acidic solvents can be sulfuric acid, acetic acid or trifluoroacetic acid. Temperature of the reaction is general between 0 and 200 °C.

Example **2** and **7** can be made by reaction of compound Example **1** with fuming sulfuric acid or a mixture of fuming sulfuric acid and concentrate sulfuric acid at temperature between 0 and 50 °C. The sulfonic acid compounds (Example **2** or **7**) can be converted to sulfonyl chloride derivatives as intermediates. Common reagents used for the chloration are sulfonyl chloride, phosphorus trichloride and phosphorus oxychloride. Amidation of the intermediates using primary or secondary amines affords target compounds (Example **3** and **4**). Formation of the final sulfonamides using sulfonylchloride with amino derivatives can be carried out by a variety of conditions known to those skilled in the art, including reaction with first or secondary amines using pyridine or triethyl amine as base. Typical solvents include chlorinated solvents, various ethers, and dipolar aprotic solvents like DMF. For example, the compounds Example **3** and **4** can be prepared by reaction of the acid Example **2** with sulfonyl chloride to give a substituted sulfonyl chloride intermediate, which was amidated with either 2-morpholin-4-yl-ethylamine or 2-piperidin-1-yl-ethylamine to give desired products.

General Procedure A (reduction of N=C bond of the compound **4**): A mixture of compound **4** (0.20 g) and $NaBH_4$ (34 mg) in 2-propanol (8 mL) was refluxed for 1h. The solvent was evaporated in vacuo and the residue was washed with water to give a desired product as a solid which could be purified by crystallization.

Example 1

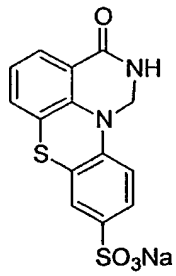
1,2-Dihydro-7-thia-2,11b-diaza-benzo[*de*]anthracen-3-one



Prepared from compound 4 according to General Procedure A. Yellowish solid, mp 187 -192 °C . ^{1} H-NMR (400 MHz, DMSO-d_6): δ 8.37 (s, 1H), 7.69-7.67 (d, 1H, J = 1.0, 8.1 Hz), 7.40-7.37 (dd, 1H, J = 1.5, 7.5 Hz), 7.30-7.24 (m, 2H), 7.14-7.10 (dd, 2H, J = 8.0, 7.6 Hz), 7.04-7.0 (dd, 1H, J = 7.6, 7.5 Hz), 5.2 (s, 2H). Anal. (C_{14} H_{10} N_2 O S· 0.2 H_2O), C H N S.

Example 2

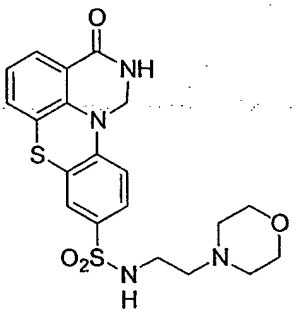
3-Oxo-2,3-dihydro-1H-7-thia-2,11b-diaza-benzo[*de*]anthracene-9-sulfonic acid sodium salt



Prepared from the Example 1 (0.12 g) and the fuming sulfuric acid at 0 ° C. Precipitation was formed upon adding ice-cold water to the mixture. The solid was collected by filtration and titrated with 2 N NaOH to give the sodium salt. mp > 300 ° C (dec). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.16 (d, 2H, J = 3.01 Hz), 7.11-7.15 (m, 1H, J = 7.53, 8.03 Hz), 7.19-7.26 (m, 2H), 7.35 (d, 1H, J = 1.51 Hz), 7.39-7.41 (dd, 1H, J = 1.50, 7.53 Hz), 7.68-7.70 (dd, 1H, J =1.51, 8.03 Hz), 8.40 (s, 1H). Anal. (C_{14} H_{10} N_2 O_4 S_2 Na2.4 H_2O) C H N S.

Example 3

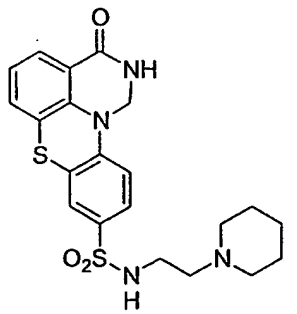
3-Oxo-2,3-dihydro-1H-7-thia-2,11b-diaza-benzo[*de*]anthracene-9-(N2-morpholin-4-yl-ethyl) sulfonamide



The acid form of Example **2** in DMF was mixed with sulfonyl chloride to give a substituted sulfonyl chloride intermediate. The solvent was removed and the residue was dissolved in a mixture of solvents methylene chloride/1,4-dioxane. To the solution was added triethyl amine and 2-morpholin-4-yl-ethylamine at 0 °C. The mixture was allowed to warm to room temperature and continued for 2 hr. The solvent was removed and the residue was purified by silica gel chromatography to give desired products. mp 227 - 228 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.49 (s,1H), 8.19 (s, 1H), 7.72 (d, 1H, J = 8.03 Hz), 7.51 - 7.48 (dd, 2H, J = 1.50, 8.01 Hz), 7.45 - 7.42 (dd, 2H, J = 1.51, 8.03 Hz), 5.27 (s, 2H), 3.94 (m, 2H), 3.78-3.72 (t, 2H, J =12.5 Hz), 3.39(m, 2H), 3.19 - 3.07 (m, 6H). Anal. (C_{20} H_{22} N_4 O_4 S_2.H_2O· 1 THF) C H N S.

Example 4

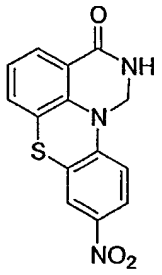
3-Oxo-2,3-dihydro-1H-7-thia-2,11b-diaza-benzo[*de*]anthracene-9-(N-2-piperidin-1-yl-ethyl)-sulfonamide



The acid form of Example **2** in DMF was mixed with sulfonyl chloride to give a substituted sulfonyl chloride intermediate. The solvent was removed and the residue was dissolved in a mixture of solvents methylene chloride/1,4-dioxane. To the solution was added triethyl amine and 2-piperidin-1-yl-ethylamine at 0 °C. The mixture was allowed to warm to room temperature and continued for 2 hr. The solvent was removed and the residue was purified by silica gel chromatography to give desired products. mp 170 - 172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (d, 1H, J = 8.03 Hz), 7.61 (s, 1H), 7.44 (d, 1H, J = 8.04 Hz), 7.25 (t, 2H, J = 8.03 Hz), 7.10 (t, 1H, J = 7.53 Hz), 5.34(s, 2H), 2.98(m, 2H), 2.35 (t, 2H, J = 6.02 Hz), 2.18 (m, 4H), 1.43 - 1.33 (m, 6H, J = 5.02 Hz). Anal. (C_{21} H_{24} N_4 O_3 S_2·0.8 H_2O. 1 C_2 H_2 O_4) C H N S.

Example 5

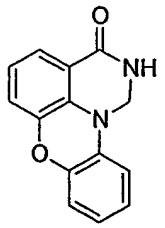
9-Nitro-1,2-dihydro-7-thia-2,11b-diaza-benzo[*de*]anthracen-3-one 5



Prepared from compound Example **1**. A mixture of the substrate in fuming nitricacid and sulfuric acid was stirred at 0 °C for 0.5 h and then poured in to ice-cold water to give a solid as a desired product. mp 205 - 208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.25 - 5.26 (d, 2H, J = 2.51 Hz), 7.07 - 7.11(t, 1H, J = 7.03 Hz), 7.18 - 7.19 (d, 1H, 3.5.Hz), 7.25 - 7.31(m, 2H), 8.13 - 8.14 (d, 1H, J = 2.51 Hz), 8.35 - 8.36 (d, 1H, J = 2.51 Hz). Anal. (C_{14} H_9 N_3 O_3 S·0.6 H_2O 0.3 acetone) C H N S.

Example 6

1,2-Dihydro-7-oxa-2,11b-diaza-benzo[*de*]anthracen-3-one

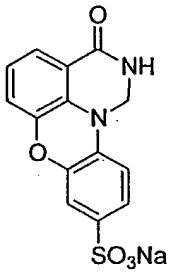


Prepared from compound **4** (X = O, R = H) according to General Procedure A. Yellow solid, mp > 300 ° C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.43 (s, 1H), 7.30 -

7.27 (dd, 1H, J = 1.5, 7.5 Hz), 6.92 -6.88 (m, 3H), 6.85 - 6.77 (m, 3H), 4.83 - 4.82 (d, 2H, J = 3.0 Hz). Anal. (C_{14} H_{10} N_2 O_2) C H N S.

Example 7

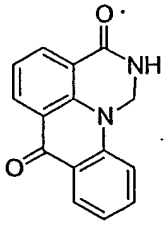
3-Oxo-2,3-dihydro-1H-7-oxa-2,11b-diaza-benzo[*de*]anthracene-9-sulfonic acid sodium salt.



Prepared from the Example **6** and the fuming sulfuric acid at 0 °C. Precipitation was formed upon adding ice-cold water to the mixture. The solid was collected by filtration and titrated with 2 N NaOH to give the sodium salt. mp 300 (dec) °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (s, 1H), 7.3 - 7.28 (dd, 1H, J = 1.5, 7.5 Hz), 7.07 - 7.04 (dd, 1H, J =1.5, 7.5 Hz), 6.92 - 6.91 (dd, 1H, J =2.0, 7.5 Hz), 6.91 - 6.88 (dd, 1H, J = 1.5, 7.5 Hz), 6.81 - 6.77 (dd, 1H, J =1.7, 8.0 Hz), 6.75 - 6.73 (d, 1H, J = 8.0 Hz), 4.81 (s, 2H). Anal. (C_{14} H_{10} N_2 O_5 S Na·0.6H_2O) C H N S.

Example 8

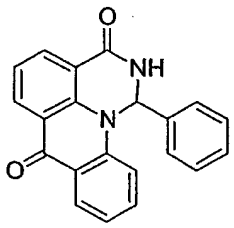
1,2-Dihydro-2,11b-diaza-benzo[*de*]anthracene-3,7-dione



Prepared from compound **4** (X = O, R = H) according to General Procedure A (0.16g, 80%). Yellow solid, mp 250 – 255 °C. MS: (M-1): 249; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, br, 1H), 8.46 (d, 1H), 8.39 - 8.32 (m, 2H), 7.90 - 7.86 (m, 2H), 7.45 - 7.40 (m, 2H), 5.71 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 176.39, 161.78, 140.87, 139.92, 134.94, 133.49, 131.41, 127.25, 122.81, 122.13, 121.77, 121.22, 118.39, 115.78, 56.46; Anal. (C_{15} H_{10} N_2 O_2·0.36 H_2O). C H N.

Example 9

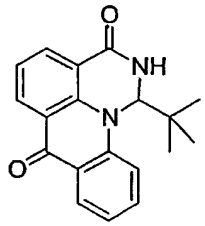
N-(5,9-Dihydro-5-oxo-4[H]cyclopenta[*lmn*]phenathridin-1-yl)-4-fluorbenzamide



A mixture of amide **3** (X = CO, 0.10 g), benzaldehyde (8 mL) and triethylamine (0.1 mL) was refluxed for 15h. The solvent was evaporated in vacuo and the residue was washed with H_2O and Et_2O to give a yellow product (0.11 g, 78%), mp 288 – 292 °C. MS: (M+1): 327; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (d, 1H), 8.53 (dd, 1H), 8.40 (dd, 1H), 8.33 (dd, 1H), 7.99 (d, 1H), 7.81 (m, 1H), 7.52 - 7.39 (m, 3H), 7.26 - 7.23 (m, 3H), 7.09-7.05 (m, 2H); $_{13}$C NMR (100 MHz, DMSO-d_6): 176.30, 161.21, 140.30, 140.27, 140.17, 135.12, 133.66, 131.90, 129.32, 129.02, 127.45, 125.76, 123.16, 122.27, 122.17, 121.37, 118.44, 116.60, 67.01; Anal. (C_{21} H_{14} N_2 O_2) C H N.

Example 10

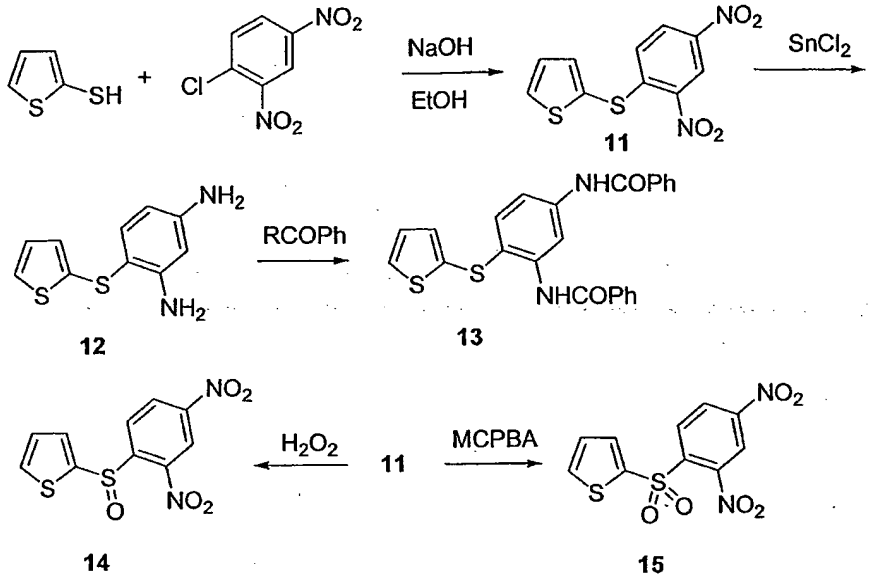
1-tert-Butyl-1,2-dihydro-2,11b-diaza-benzo[*de*]anthracene-3,7-dione



A mixture of amide **3** (X = CO, 0.10 g), trimethylacetaldehyde (8 mL) and triethylamine (0.1 mL) was refluxed for 15h. The solvent was evaporated in vacuo and the residue was washed with H_2O and Et_2O to give a yellow product (0.12 g, 92%), mp 300 °C dec. MS: (M+1): 307; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (d, 1H), 8.43 (dd, 1H), 8.33 (dd, 1H), 8.28 - 8.22 (m, 2H), 7.81 (m, 1H), 7.43 (t, 1H), 7.37 (t, 1H), 6.25 (d, 1H), 0.68 (s, 9H); Anal. (C_{19} H_{18} N_2 O_2· 0.2 H_2O) C H N.

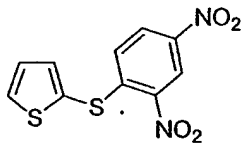
2-(2,4-Dinitro-phenylsulfanyl)-thiophene derivatives of this invention are represented by previously defined formula II. As an example, these derivatives can be prepared in a conventional manner as illustrated below by Scheme 2.

Scheme 2



Example 11

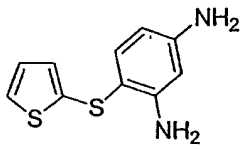
2-(2,4-Dinitro-phenylsulfanyl)-thiophene



To a solution of thiophene-2-thiol (0.57 g, 4.9 mmol) in EtOH (15 mL) was added NaOH (0.3 g, 4.9 mmol), which was dissolved in 1.5 mL of water. Then to a solution 2,4-dinitrochlorobenzene (1.0 g, 4.9 mmol) in EtOH (15 mL) was added the previous solution while stirring. At this point mercaptans react almost instantly as shown by the generation of heat and the precipitation of the sulfide. To ensure complete reaction the mixture was heated to reflux for 15 minutes. The hot solution was quickly filtered, whereupon the sulfide crystallized in golden-yellow needles from the filtrate on cooling. The pure product (1.2 g, 86%) was obtained from re-crystallization in absolute alcohol. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.13 (d, J = 9.0 Hz, 1H), 7.27 (t, J = 3.7 Hz, 1H), 7.46 (d, J = 3.6 Hz, 1H), 7.75 (d, J = 5.3 Hz, 1H), 8.22 (dd, J = 9.0 and 2.4 Hz, 1H), 9.09 (d, J = 2.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): 121.60, 126.82, 127.59, 129.08, 129.66, 134.92, 139.36, 144.26, 145.12, 148.83. MS: 282. Anal: ($C_{10}H_6N_2O_4S_2$) C H N S. Physical Form: Yellow solid.

Example 12

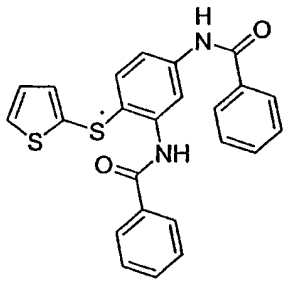
4-(Thiophen-2-ylsulfanyl)-benzene-1,3-diamine



To a solution of 2-(2,4-Dinitro-phenylsulfanyl)-thiophene (0.50 g, 1.8 mmol) in EtOAc (25 mL) was added $SnCl_2 2H_2O$ (2.8 g, 12.4 mmol) while stirring. The mixture was heated to reflux for 3 hrs. The mixture was cooled to room temperature and sat. $NaHCO_3$ was added until slightly basic. Another 15 mL of EtOAc was added and the organic layer was separated. The aqueous layer was extracted by 40 mL of EtOAc. The combined organic layers were washed by water and brine, then was dried over $MgSO_4$. The removal of the solvents gave an oil (0.21g, 84%) as product. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.28 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 8.4, 2.3 Hz, 1H), 7.00 (dd, J = 5.2 and 1.2 Hz, 1H), 6.89 (t, J = 3.9 Hz, 1H), 6.09 – 6.03 (m, 2H), 4.31 (s, br, 2H), 3.69 (s, br, 2H). ^{13}C NMR ($CDCl_3$): 149.35, 149.28, 137.88, 137.84, 128.74, 127.31, 126.86, 106.90, 106.53, 100.94. MS: 222. Anal: ($C_{10}H_{10}N_2S_2$) C H N S. Physical Form: dark oil.

Example 13

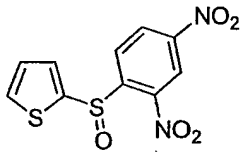
N-[4-(thiophen-2-ylsulfanyl)-phenyl]-1,3-benzamide



To a solution of 4-(Thiophen-2-ylsulfanyl)-benzene-1,3-diamine (0.10 g, 0.45 mmol) in CH_2Cl_2 (4 mL) was added benzoyl chloride (0.11 mL, 0.95 mmol) and N,N-diisopropylethylamine (0.33 mL, 1.9 mmol). The mixture was stirred for 3 hrs. The solvent was removed and the residue was purified by silica gel chromatography to give desired off-white solid (0.15 g, 79%) as product. ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.30 (s, 1H), 8.57 (s, 1H), 8.10 – 8.03 (m, 2H), 7.92 – 7.87 (m, 4H), 7.68 (d, J = 8.4 Hz, 1H), 7.60 – 7.47 (m, 6H), 7.28 (dd, J = 5.0, 1.0 Hz, 1H), 7.11 (d, J = 5.1 Hz, 1H), 6.94 (t, J = 5.2 Hz, 1H). MS: 431. Anal: ($C_{24}H_{18}N_2O_2S_2$) C H N S. Physical Form: off-white solid.

Example 14

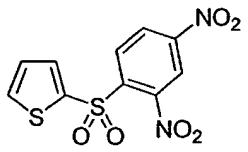
2-(2,4-Dinitro-benzenesulfinyl)-thiophene



To a solution of 2-(2,4-Dinitro-phenylsulfanyl)-thiophene (0.20 g, 0.71 mmol) in acetic acid (2 mL) was added 30 % H_2O_2 (0.30 mL, 3.5 mmol). The mixture was heated to 100 °C for 1 hr. The solvent was removed and the residue was purified by silica gel chromatography to give desired yellow solid (0.18 g, 86%) as product. ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.09 (s, 1H), 8.81 (d, J = 1.1 Hz, 2H), 7.72 (dd, J = 4.8, 1.0 Hz, 1H), 7.57 (dd, J = 5.1, 1.0 Hz, 1H), 7.08 (t, J = 5.1 Hz, 1H). MS: 298. Anal: ($C_{10}H_6N_2O_5S_2$) C H N S. Physical Form: yellow solid.

Example 15

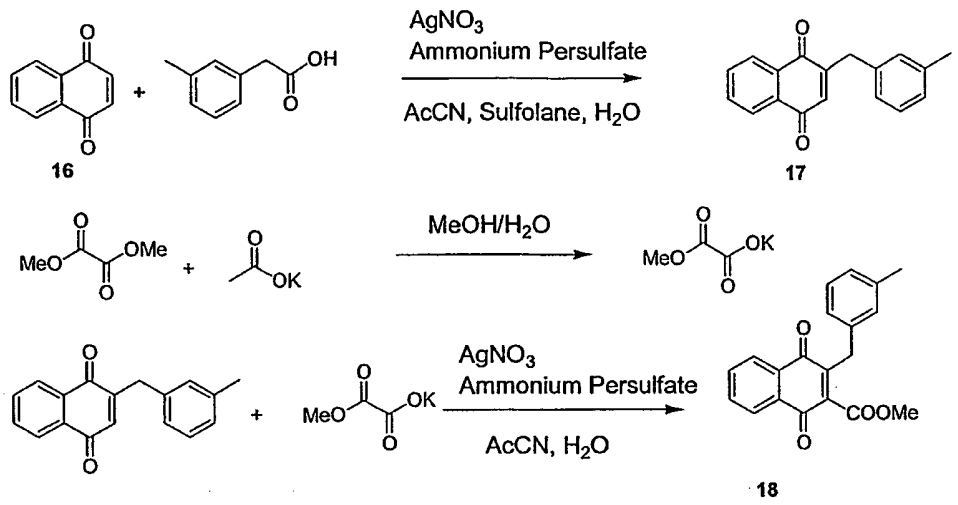
2-(2,4-Dinitro-benzenesulfonyl)-thiophene



To a solution of 2-(2,4-Dinitro-phenylsulfanyl)-thiophene (0.10 g, 0.35 mmol) in AcOH/CH_2Cl_2 (2/2 mL) was added 3-chloroperoxybenzoic acid (77 % max. 0.6 g mL, excess). The mixture was heated to reflux for 8 hrs. The mixture was cooled and the solvent was removed and the residue was purified by silica gel chromatography to give desired yellow solid (0.10 g, 81 %) as product. ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.60 –8.40 (m, 3H), 7.98 (dd, J = 4.9, 1.0 Hz, 1H), 7.85 (dd, J = 5.0, 1.1 Hz, 1H), 7.22 (t, J = 4.9 Hz, 1H). MS: 314. Anal: ($C_{10}H_6N_2O_6S_2$) C H N S. Physical Form: yellow solid.

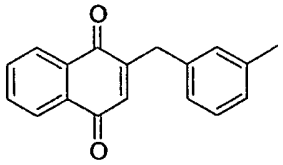
1,4-Naphthoquinone derivatives of this invention are represented by previously defined formula III. As an example, these derivatives can be prepared in a conventional manner as illustrated below by Scheme 3.

Scheme 3



Example 16

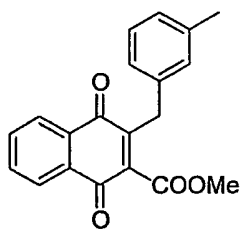
2-(3-Methyl-benzyl)-[1,4]-naphthoquinone



To a solution of 1,4-naphthoquinone (1.0 g, 6.3 mmol), m-tolylacetic acid (0.95 g, 6.3 mmol) in AcCN (7 mL), water (47 mL) and sulfolane (21 mL) was added silver nitrate (0.5 g, 2.9 mmol). The reaction mixture was heated to 60-65 °C until complete dissolution. Then ammonium persulfate (1.7 g) in water was added slowly. The reaction was stirred at 60-65 °C for another 5 hours and cooled down to room temperature. The solution was extracted with 80 mL of EtOAc twice. Organic layers were combined, washed with brine and dried. Solvent was remove and the residue was purified using column chromatography to give a product (1.2 g, 72 %). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.34 (s, 3H), 3.86 (s, 2H), 7.05-7.15 (m, 3H), 7.20-7.25 (m, 2H), 7.70-7.80 (m, 2H), 8.00-8.20 (m, 2H). Physical Form: Yellow solid.

Example 17

3-(3-Methyl-benzyl)-1,4-dioxo-1,4-dihydro-naphthalene-2-carboxylic acid methyl ester



Preparation of potassium monomethyloxalate: 1.2 g of dimethyloxalate and 0.98 g of potassium acetate were dissolved in 10 mL of MeOH and 10mL of water. The solution was stirred at 80 °C for 3 hours. Solvent was removed. A white solid was obtained (quantitative yield).

The compound 18 was made in the same fashion as the compound 17 was made except that the reaction was stirred at higher temperature (89 - 90 °C). A product was obtained with 20 % yield. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.31 (s, 3H), 3.89 (s, 2H), 3.94 (s, 3H), 6.95-7.20 (m, 4H), 7.70-7.85 (m, 2H), 8.00 - 8.25 (m, 2H). Physical Form: Yellow oil.

Example 18

Neuroprotective Effect of DPQ on Focal Cerebral Ischemia in Rats

Focal cerebral ischemia can be produced by cauterization of the right distal MCA (middle cerebral artery) with bilateral temporary common carotid artery occlusion in male Long-Evans rats for 90 minutes. Two hours prior to MCA occlusion, varying amounts (control, n=14; 5 mg/kg, n=7; 10 mg/kg, n=7; 20 mg/kg, n=7; and 40 mg/kg, n=7) of the compound can be dissolved in dimethyl sulfoxide (DMSO) using a sonicator. A volume of 1.28 ml/kg of the resulting solution can be injected intraperitoneally into the rats.

The rats then can be anesthetized with halothane (4% for induction and 0.8%-1.2% for the surgical procedure) in a mixture of 70% nitrous oxide and 30% oxygen. The body temperature can be monitored by a rectal probe and maintained at 37.5.+-.0.5.degree. C. with a heating blanket regulated by a homeothermic blanket control unit (Harvard Apparatus Limited, Kent, U.K.). A catheter (PE-50) can be placed into the tail artery, and arterial pressure can be continuously monitored and recorded on a Grass polygraph recorder (Model 7D, Grass Instruments, Quincy, Mass.): Samples for blood gas analysis (arterial pH, PaO.sub.2 and PaCO.sub.2) can also be taken from the tail artery catheter and measured with a blood gas analyzer (ABL 30, Radiometer, Copenhagen, Denmark). Arterial blood samples can be obtained 30 minutes after MCA occlusion.

The head of the animal can be positioned in a stereotaxic frame, and a right parietal incision between the right lateral canthus and the external auditory meatus can be made. Using a dental drill constantly cooled with saline, a 3 mm burr hole can be prepared over the cortex supplied by the right MCA, 4 mm lateral to the sagittal suture and 5 mm caudal to the coronal suture. The dura mater and a thin inner bone layer can be kept, care being taken to position the probe over a tissue area devoid of large blood vessels. The flow probe (tip diameter of 1 mm, fiber separation of 0.25 mm) can be lowered to the bottom of the cranial burr hole using a micromanipulator. The probe can be held stationary by a probe holder secured to the skull with dental cement. The microvascular blood flow in the right parietal cortex can be continuously monitored with a laser Doppler flowmeter (FloLab, Moor, Devon, U.K., and Periflux 4001, Perimed, Stockholm, Sweden).

Focal cerebral ischemia can be produced by cauterization of the distal portion of the right MCA with bilateral temporary common carotid artery (CCA) occlusion by the procedure of Chen et al., "A Model of Focal Ischemic Stroke in the Rat: Reproducible Extensive Cortical Infarction", Stroke 17:738-43 (1986) and/or Liu et al., "Polyethylene Glycol-conjugated Superoxide Dismutase and Catalase Reduce Ischemic Brain Injury", Am. J. Physiol. 256:H589-93 (1989), both of which are hereby incorporated by reference.

Specifically, bilateral CCA's can be isolated, and loops made from polyethylene (PE-10) catheter can be carefully passed around the CCA's for later remote occlusion. The incision made previously for placement of the laser doppler probe can be extended to allow observation of the rostral end of the zygomatic arch at the fusion point using a dental drill, and the dura mater overlying the MCA can be cut. The MCA distal to its crossing with the inferior cerebral vein can be lifted by a fine stainless steel hook attached to a micromanipulator and, following bilateral CCA occlusion, the MCA can be cauterized with an electrocoagulator. The burr hole can be covered with a small piece of Gelform, and the wound can be sutured to maintain the brain temperature within the normal or near-normal range.

After 90 minutes of occlusion, the carotid loops can be released, the tail arterial catheter can be removed, and all of the wounds can be sutured. Gentamicin sulfate (10 mg/ml) can be topically applied to the wounds to prevent infection. The anesthetic can be discontinued, and the animal can be returned to his cage after awakening. Water and food can be allowed ad libitum.

Two hours after MCA occlusion, the animals can be given the same doses of the compound as in the pretreatment. Twenty-four hours after MCA occlusion, the rats can be sacrificed with an intraperitoneal injection of pentobarbital sodium (150 mg/kg). The brain can be carefully removed from the skull and cooled in ice-cold artificial CSF for five minutes. The cooled brain can then be sectioned in the coronal plane at 2 mm intervals using a rodent brain matrix (RBM-4000C, ASI Instruments, Warren, Michigan). The brain slices can be incubated in phosphate-buffered saline containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37.degree. C. for ten minutes. Color photographs can be taken of the posterior surface of the stained slices and can be used to determine the damaged area at each cross-sectional level using a computer-based image analyzer (NIH Image 1.59). To avoid artifacts due to edema, the damaged area can be calculated by subtracting the area of the normal tissue in the hemisphere ipsilateral to the stroke from the area of the hemisphere contralateral to the stroke, by the method of Swanson et al., "A Semiautomated Method for Measuring Brain Infarct Volume", J. Cereb. Blood Flow Metabol. 10:290-93 (1990), the disclosure of which is hereby incorporated by reference. The total volume of infarction can be calculated by summation of the damaged volume of the brain slices.

The cauterization of the distal portion of the right MCA with bilateral temporary CCA occlusion can consistently produce a well-recognized cortical infarct in the right MCA territory of each test animal. There can be an apparent uniformity in the distribution of the damaged area as measured by TTC staining in each group.

Example 19

Retinal Ischemia Protection

A patient just diagnosed with acute retinal ischemia is immediately administered parenterally, either by intermittent or continuous intravenous administration, a compound as disclosed herein, either as a single dose or a series of divided doses of the compound. After this initial treatment, and depending on the patient's presenting neurological symptoms, the patient optionally may receive the same or a different compound of the invention in the form of another parenteral dose. It is expected by the inventors that significant prevention of neural tissue damage would ensue and that the patient's neurological symptoms would considerably lessen due to the administration of the compound, leaving fewer residual neurological effects post-stroke. In addition, it is expected that the re-occurrence of retinal ischemia would be prevented or reduced.

Example 20

Treatment of Retinal Ischemia

A patient has just been diagnosed with acute retinal ischemia. Immediately, a physician or a nurse parenterally administers a compound as disclosed herein, either as a single dose or as a series of divided doses. The patient also receives the same or a different compound by intermittent or continuous administration via implantation of a biocompatible, biodegradable polymeric matrix delivery system comprising a compound as disclosed herein, or via a subdural pump inserted to administer the compound directly to the infarct area of the brain. It is expected by the inventors that the patient would awaken from the coma more quickly than if the compound of the invention were not administered. The treatment is also expected to reduce the severity of the patient's residual neurological symptoms. In addition, it is expected that re-occurrence of retinal ischemia would be reduced.

Example 21

Assay for Neuroprotective Effects on Focal Cerebral Ischemia in Rats

Focal cerebral ischemia experiments can be performed using male Wistar rats weighing 250-300 g, which can be anesthetized with 4% halothane. Anesthesia can be maintained with 1.0-1.5% halothane until the end of surgery. The animals can be installed in a warm environment to avoid a decrease in body temperature during surgery.

An anterior midline cervical incision can be made. The right common carotid artery (CCA) can be exposed and isolated from the vagus nerve. A silk suture can be placed and tied around the CCA in proximity to the heart. The external carotid artery (ECA) can then be exposed and ligated with a silk suture. A puncture can be made in the CCA and a small catheter (PE 10, Ulrich & Co., St-Gallen, Switzerland) can be gently advanced to the lumen of the internal carotid artery (ICA). The pterygopalatine artery may not be occluded. The catheter can be tied in place with a silk suture. Then, a 4-0

nylon suture (Braun Medical, Crissier, Switzerland) can be introduced into the catheter lumen and can be pushed until the tip blocks the anterior cerebral artery. The length of catheter into the ICA can be approximately 19 mm from the origin of the ECA. The suture can be maintained in this position by occlusion of the catheter with heat. One cm of catheter and nylon suture can be left protruding so that the suture can be withdrawn to allow reperfusion. The skin incision can be closed with wound clips.

The animals can be maintained in a warm environment during recovery from anesthesia. Two hours later, the animals can be re-anesthetized, the clips can be discarded, and the wound can be re-opened. The catheter can be cut, and the suture can be pulled out. The catheter can then be obturated again by heat, and wound clips can be placed on the wound. The animals can be allowed to survive for 24 hours with free access to food and water. The rats can be sacrificed with CO.sub.2 and decapitated.

The brains can be immediately removed, frozen on dry ice and stored at -80.degree. C. The brains can be cut in 0.02 mm-thick sections in a cryocut at -19.degree. C., selecting one of every 20 sections for further examination. The selected sections can be stained with cresyl violet according to the Nissl procedure. Each stained section can be examined under a light microscope, and the regional infarct area can be determined according to the presence of cells with morphological changes.

Various doses of the compounds of the invention can be tested in this model. The compounds can be administered in either a single dose or a series of multiple doses, i.p. or i.v., at different times, both before or after the onset of ischemia. Compounds of the invention can be found to provide protection from ischemia in the range of about 20 to 80%.

Example 22

Effects on Heart Ischemia/Reperfusion Injury in Rats

Female Sprague-Dawley rats, each weighing about 300-350 g can be anesthetized with intraperitoneal ketamine at a dose of 150 mg/kg. The rats can be endotracheally intubated and ventilated with oxygen-enriched room air using a Harvard rodent ventilator. Polyethylene catheters inserted into the carotid artery and the femoral vein are used for artery blood pressure monitoring and fluid administration respectively. Arterial pCO.sub.2 can be maintained between 35 and 45 mm Hg by adjusting the respirator rate. The rat chests can be opened by median sternotomy, the pericardium can be incised, and the hearts can be cradled with a latex membrane tent. Hemodynamic data can be obtained at baseline after at least a 15-minute stabilization period following the end of the surgical operation. The LAD (left anterior descending) coronary artery can be ligated for 40 minutes, and then re-perfused for 120 minutes. After 120 minutes' reperfusion, the LAD artery can be re-occluded, and a 0.1 ml bolus of monastral blue dye can be injected into the left atrium to determine the ischemic risk region.

The hearts can be arrested with potassium chloride and cut into five 2-3 mm thick transverse slices. Each slice can be weighed and incubated in a 1% solution of trimethyltetrazolium chloride to visualize the infarcted myocardium located within the

risk region. Infarct size can be calculated by summing the values for each left ventricular slice and can be further expressed as a fraction of the risk region of the left ventricle.

Various doses of the compounds of the invention can be tested in this model. The compounds can be given either in a single dose or a series of multiple doses, i.p. or i.v., at different times, both before or after the onset of ischemia. The compounds of the invention can be found to have ischemia/reperfusion injury. Therefore, they can protect against ischemia-induced degeneration of rat hippocampal neurons in vitro.

Example 23

Vascular Stroke Protection

A patient just diagnosed with acute vascular stroke is immediately administered parenterally, either by intermittent or continuous intravenous administration, a compound as disclosed herein, either as a single dose or a series of divided doses of the compound. After this initial treatment, and depending on the patient's presenting neurological symptoms, the patient optionally may receive the same or a different compound of the invention in the form of another parenteral dose. It is expected by the inventors that significant prevention of neural tissue damage would ensue and that the patient's neurological symptoms would considerably lessen due to the administration of the compound, leaving fewer residual neurological effects post-stroke. In addition, it is expected that the re-occurrence of vascular stroke would be prevented or reduced.

Example 24

Treatment of Vascular Stroke

A patient has just been diagnosed with acute multiple vascular strokes and is comatose. Immediately, a physician or a nurse parenterally administers a compound as disclosed herein, either as a single dose or as a series of divided doses. Due to the comatose state of the patient, the patient also receives the same or a different compound by intermittent or continuous administration via implantation of a biocompatible, biodegradable polymeric matrix delivery system comprising a compound as disclosed herein, or via a subdural pump inserted to administer the compound directly to the infarct area of the brain. It is expected by the inventors that the patient would awaken from the coma more quickly than if the compound of the invention were not administered. The treatment is also expected to reduce the severity of the patient's residual neurological symptoms. In addition, it is expected that re-occurrence of vascular stroke would be reduced.

Example 25

Preventing Cardiac Reperfusion Injury

A patient is diagnosed with life-threatening cardiomyopathy and requires a heart transplant. Until a donor heart is found, the patient is maintained on Extra Corporeal Oxygenation Monitoring (ECMO).

A donor heart is then located, and the patient undergoes a surgical transplant procedure, during which the patient is placed on a heart-lung pump. The patient receives a compound as disclosed herein intracardiac within a specified period of time prior to re-routing his or her circulation from the heart-lung pump to his or her new heart, thus preventing cardiac reperfusion injury as the new heart begins to beat independently of the external heart-lung pump.

Example 26

In vitro Radiosensitization

The human prostate cancer cell line, PC-3s, can be plated in 6 well dishes and grown at monolayer cultures in RPMIl640 supplemented with 10% FCS. The cells are maintained at 37.degree. C. in 5% CO.sub.2 and 95% air. The cells were exposed to a dose response (0.1 mM to 0.1 uM) of 3 different compounds as disclosed herein prior to irradiation at one sublethal dose level. For all treatment groups, the six well plates can be exposed at room temperature in a Seifert 250 kV/15 mA irradiator with a 0.5 mm Cu/l mm. Cell viability can be examined by exclusion of 0.4% trypan blue. Dye exclusion can be assessed visually by microscopy and viable cell number can be calculated by subtracting the number of cells from the viable cell number and dividing by the total number of cells. Cell proliferation rates can be calculated by the amount of .sup.3H-thymidine incorporation post-irradiation.

Example 27

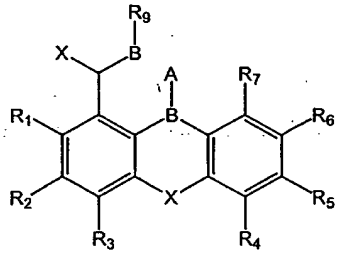
In vivo Radiosensitization

Before undergoing radiation therapy to treat cancer, a patient is administered an effective amount of a compound or a pharmaceutical composition of the present invention. The compound or pharmaceutical composition acts as a radiosensitizer and making the tumor more susceptible to radiation therapy.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications are intended to be included within the scope of the following claims.

We claim:

1. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said inhibitor is a compound of formula I:

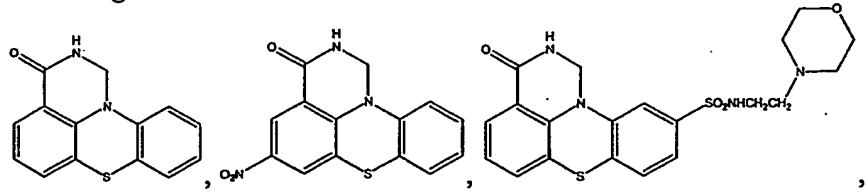


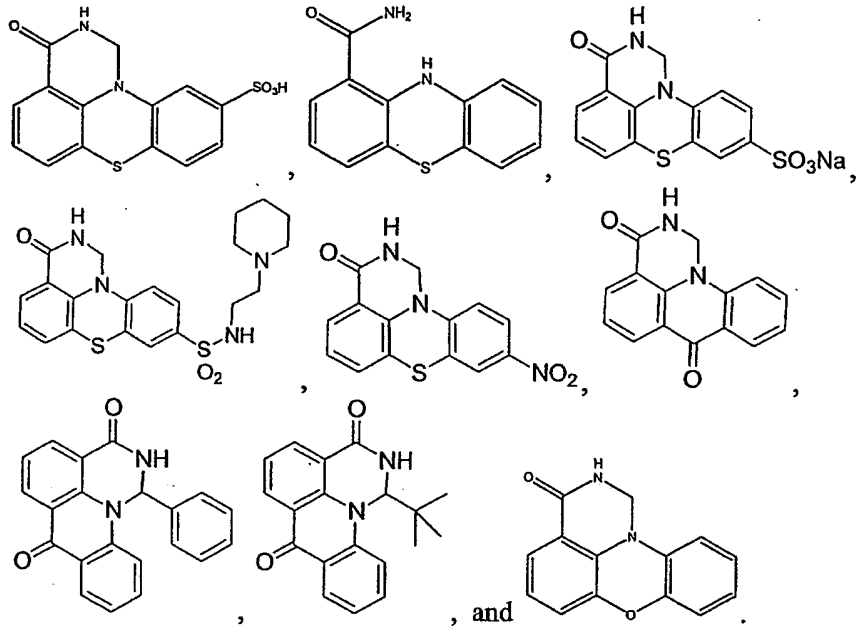
or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

wherein:
X is O, N, S, P, C=O;
A is a bond, H, CH2, CHR8, -CH2-NH-, -CHR8-NH-, -CH2-NR8-, -(CH2)2-, -CH2-CHR8-;
B is C, N, S, C-A, N-A, S-A; and
R1, R2, R3, R4, R5, R6, R7, R8, R9 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

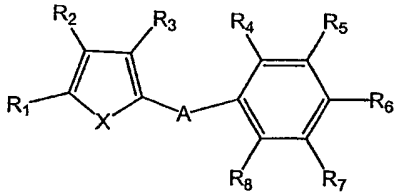
2. A method of claim 1, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

3. A method of claim 1, wherein the compound is selected from the group consisting of





4. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said inhibitor is a compound of formula II:

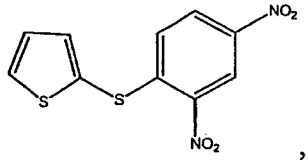


or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

wherein:
X is O, N, S, P;
A is O, S, S=O, SO2, CH2, CHR9, -CH2-NH-, -CHR9-NH-, -CH2-NR9-, -(CH2)2-, -CH2-CHR9-; and
R1, R2, R3, R4, R5, R6, R7, R8, R9 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

5. A method of claim 4, wherein, R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

6. A method of claim 4, wherein the compound is selected from the group consisting of: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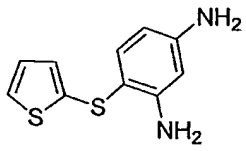
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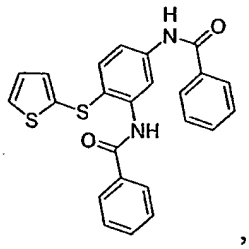
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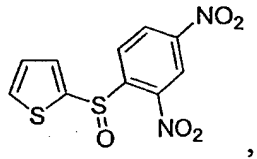
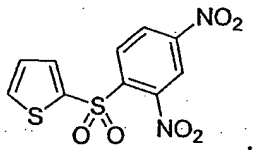
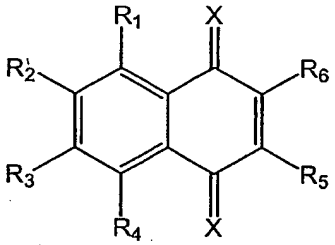

, and

.

7. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of formula III:

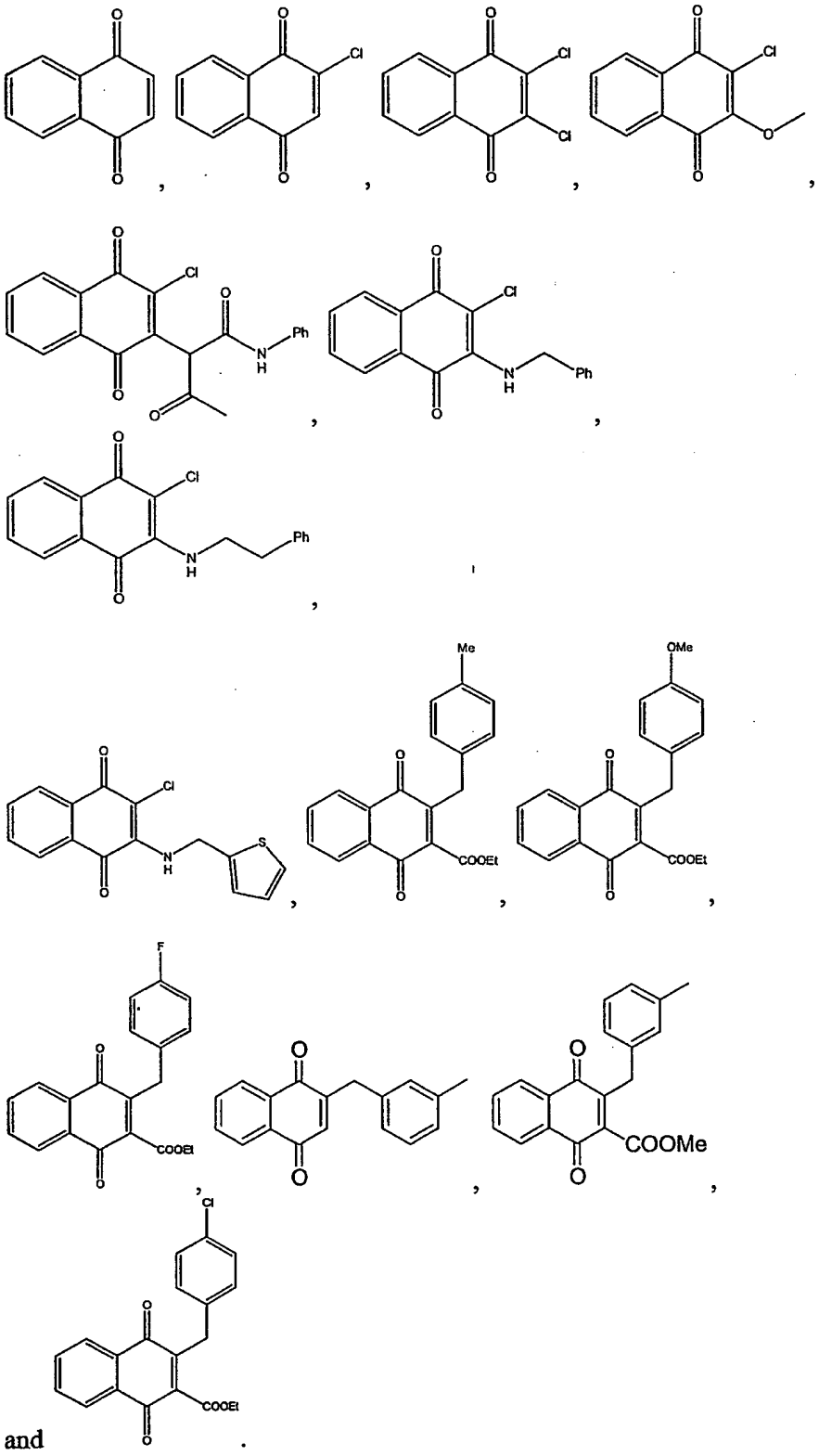


or a pharmaceutically acceptable salt, hydrate, metabolite, or prodrug,

wherein:
X is O, N, S, P; and
R1, R2, R3, R4, R5, R6 is an optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, -OH an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, or –OR11 or –N11R12 where R11 and R12 are each independently hydrogen or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

8. A method of claim 7, wherein, R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

9. A method of claim 7, wherein the compound is selected from the group consisting of:



and .

10. A method of claim 1, 4, or 7 wherein the cancer is selected from the group consisting of bladder cancer, brain cancer, breast cancer, cervical cancer, head and

neck cancer, Hodgkin's lymphoma, lung cancer (small and/or non-small cell), melanoma, non-Hodgkin's lymphoma, ovarian cancer, prostrate cancer, skin cancer, and mixtures thereof.

11. A method of claim 10 wherein the cancer comprises bladder cancer.

12. A method of claim 10 wherein the cancer comprises brain cancer.

13. A method of claim 10 wherein the cancer comprises breast cancer.

14. A method of claim 10 wherein the cancer comprises cervical cancer.

15. A method of claim 10 wherein the cancer comprises head and neck cancer.

16. A method of claim 10 wherein the cancer comprises Hodgkin's lymphoma.

17. A method of claim 10 wherein the cancer comprises lung cancer (small and/or non-small cell).

18. A method of claim 10 wherein the cancer comprises melanoma.

19. A method of claim 10 wherein the cancer comprises non-Hodgkin's lymphoma.

20. A method of claim 10 wherein the cancer comprises ovarian cancer.

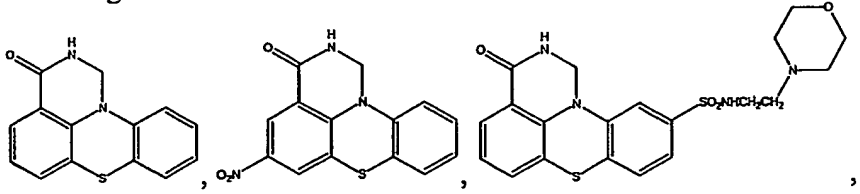
21. A method of claim 10 wherein the cancer comprises prostrate cancer.

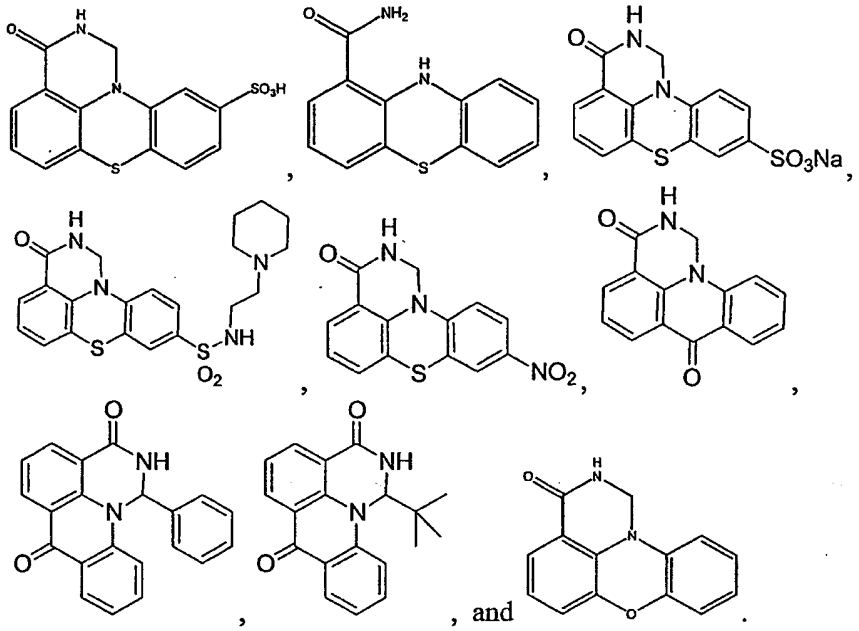
22. A method of claim 10 wherein the cancer comprises skin cancer.

23. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and an anti-cancer agent to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 1:

24. A method of claim 23, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

25. A method of claim 23, wherein the compound is selected from the group consisting of

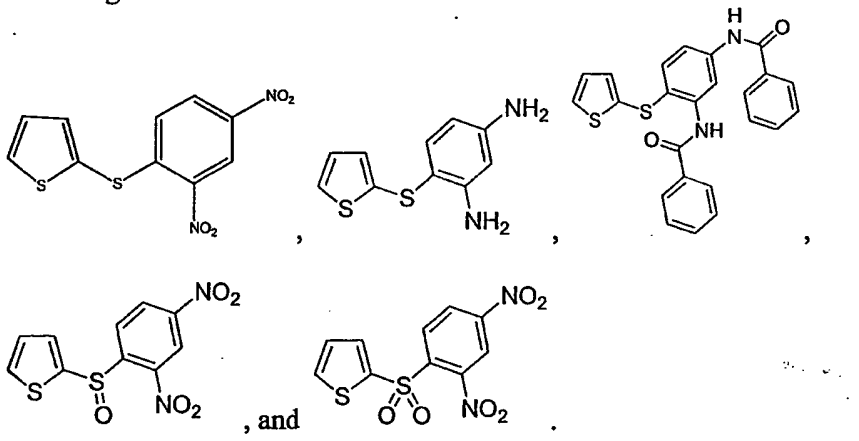




26. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and an anti-cancer agent to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 4.

27. A method of claim 26, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

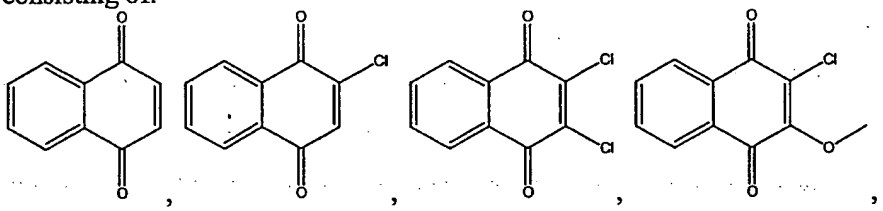
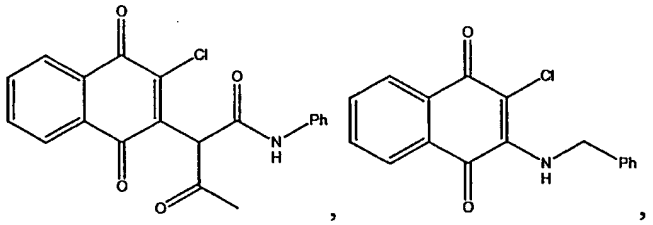
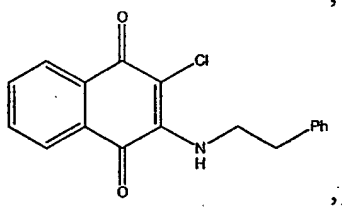
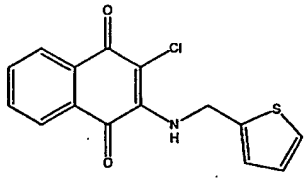
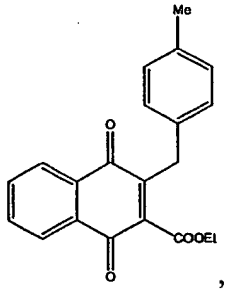
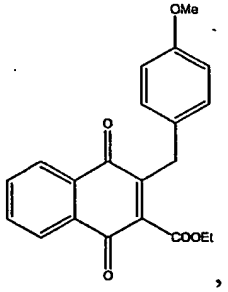
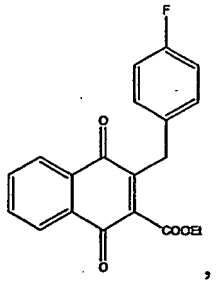
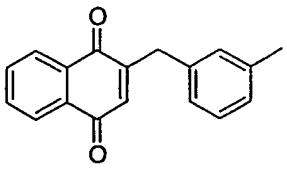
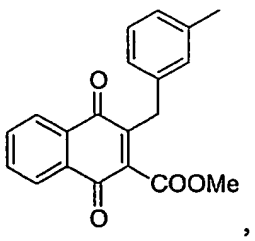
28. A method of claim 26, wherein the compound is selected from the group consisting of:

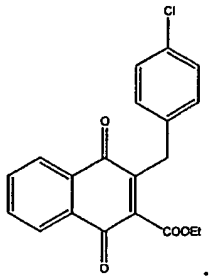


29. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and an anti-cancer agent to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 7.

30. A method of claim 29, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

31. A method of claim 29, wherein the compound is selected from the group consisting of:



and .

32. A method of claim 23, 26, or 29 wherein the anti-cancer agent is selected from the group consisting of chemotherapy, radiosensitizers, and mixtures thereof.

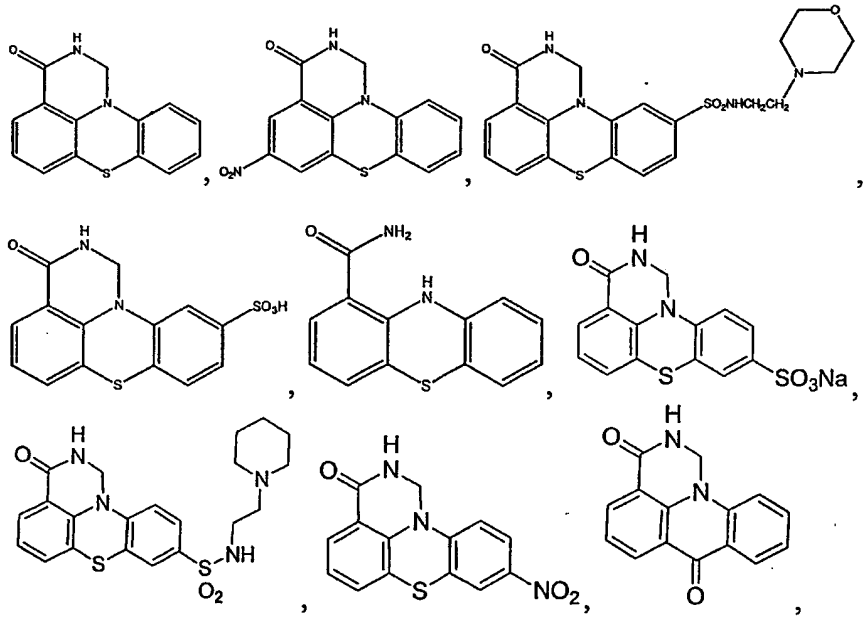
33. A method of claim 32 wherein the anti-cancer agent comprises chemotherapy.

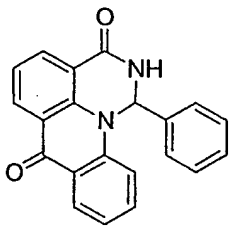
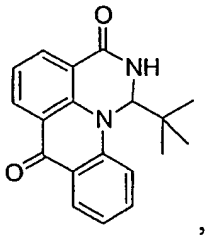
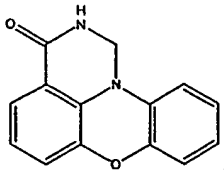
34. A method of claim 32 wherein the anti-cancer agent comprises radiosensitizers.

35. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a cardiovascular disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 1.

36. A method of claim 35, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

37. A method of claim 35, wherein the compound is selected from the group consisting of...

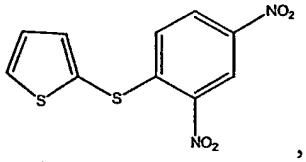
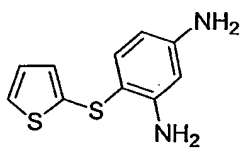
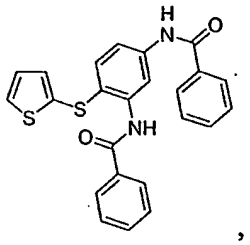
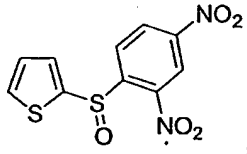
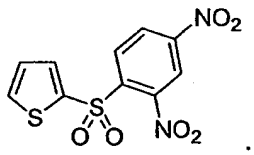


38. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a cardiovascular disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 4.

39. A method of claim 38, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

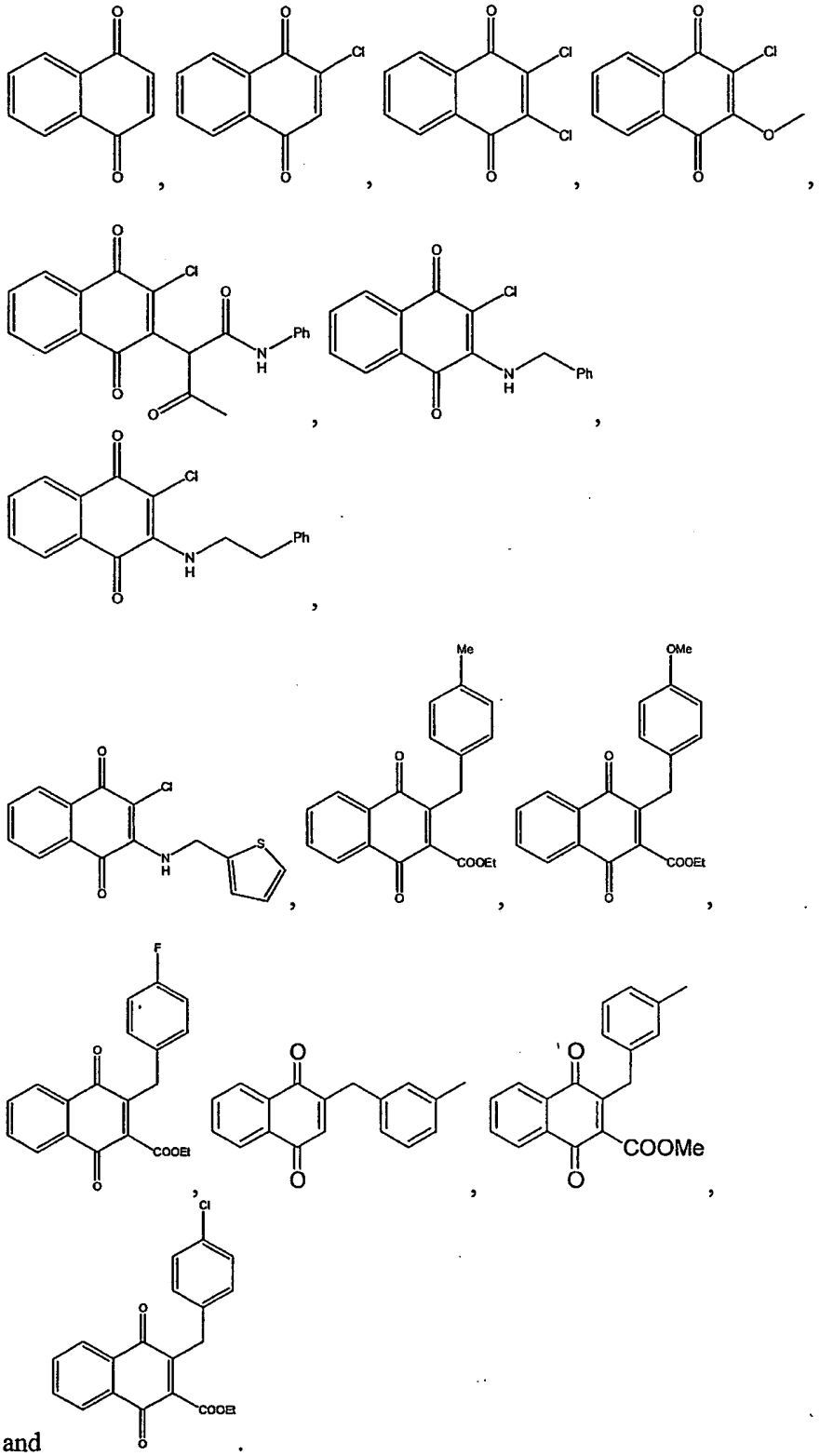
40. A method of claim 38, wherein the compound is selected from the group consisting of

41. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a cardiovascular disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 7.

42. A method of claim 41, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

43. A method of claim 41, wherein the compound is selected from the group consisting of



and .

44. A method of claim 35, 38, or 41 wherein the cardiovascular disorder is selected from the group consisting of cardiovascular tissue damage, coronary

artery disease, myocardial infarction, angina pectoris, cardiogenic shock, coronary artery bypass surgery, cardiac arrest, cardio-pulmonary resuscitation, and mixtures thereof.

45. A method of claim 44 wherein the cardiovascular disorder comprises cardiovascular tissue damage.

46. A method of claim 44 wherein the cardiovascular disorder comprises coronary artery disease.

47. A method of claim 44 wherein the cardiovascular disorder comprises myocardial infarction.

48. A method of claim 44 wherein the cardiovascular disorder comprises angina pectoris.

49. A method of claim 44 wherein the cardiovascular disorder comprises cardiogenic shock.

50. A method of claim 44 wherein the cardiovascular disorder comprises coronary artery bypass surgery.

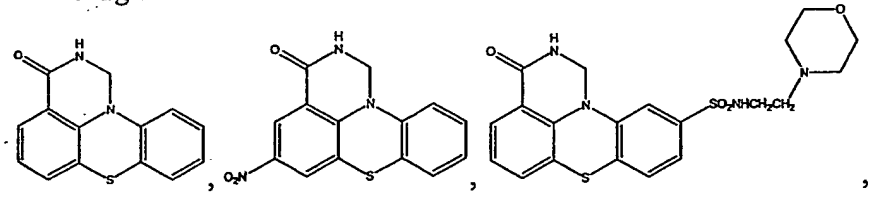
51. A method of claim 44 wherein the cardiovascular disorder comprises cardiac arrest.

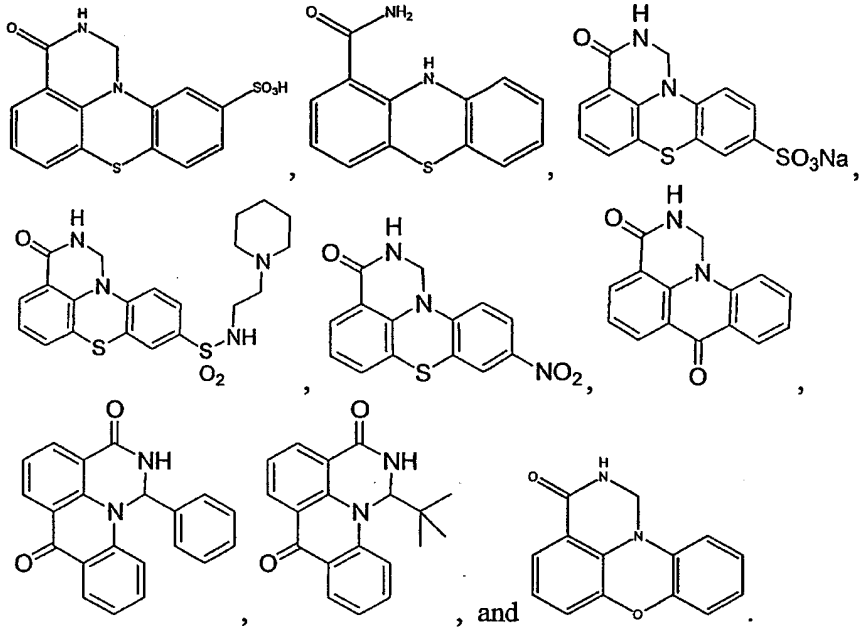
52. A method of claim 44 wherein the cardiovascular disorder comprises cardio-pulmonary resuscitation.

53. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 1.

54. A method of claim 53, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

55. A method of claim 53, wherein the compound is selected from the group consisting of

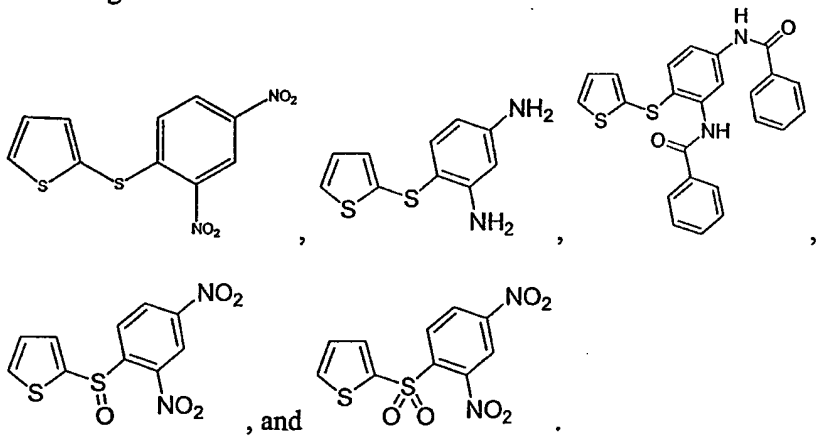




56. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 4.

57. A method of claim 56, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

58. A method of claim 56, wherein the compound is selected from the group consisting of:

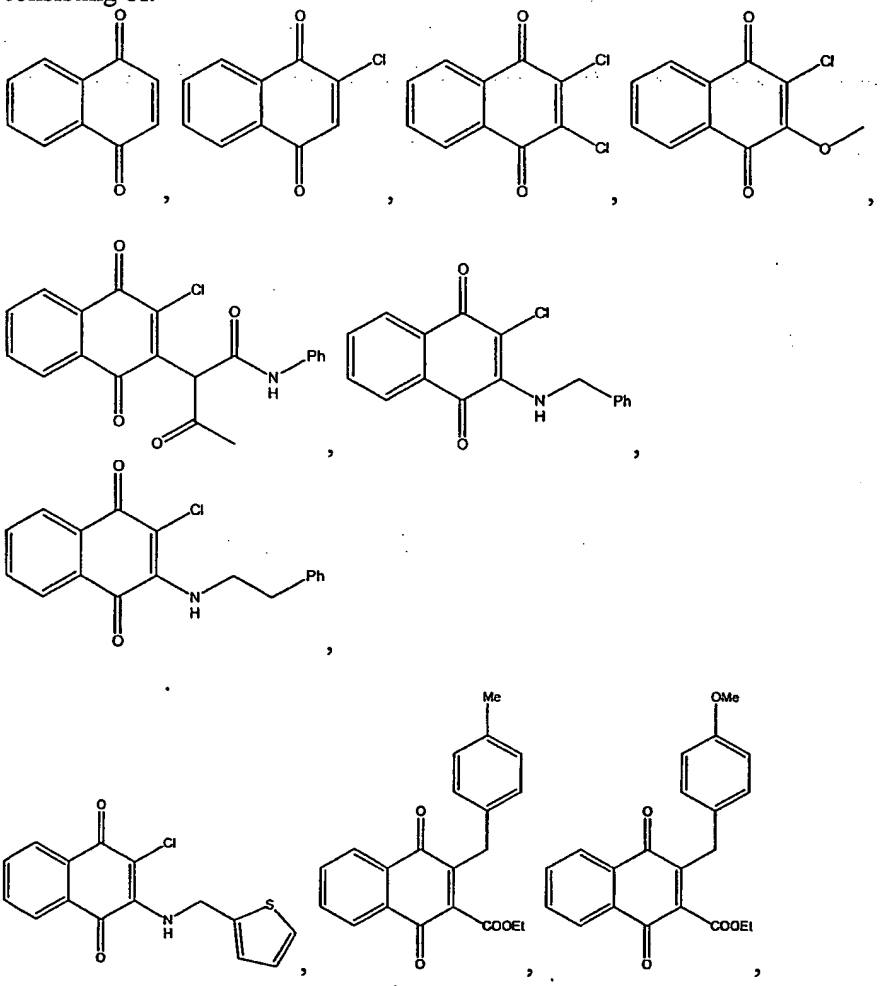


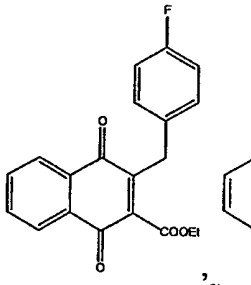
59. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor and a Type I or Type II histone deacetylase

inhibitor to treat cancer wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 7.

60. A method of claim 59, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

61. A method of claim 59, wherein the compound is selected from the group consisting of:





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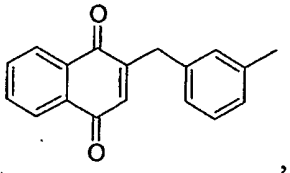

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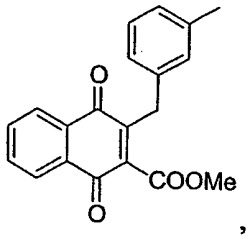

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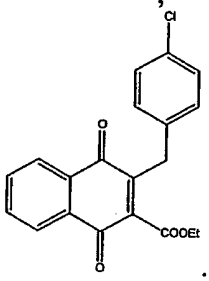


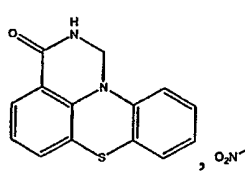
and .

62. A method of claim 53, 56, or 59, wherein the Type I or Type II histone deacetylase inhibitor is selected from the group consisting of tricostatin A, SAHA, oxamflatin, trapoxin A, FR901228, apicidin, MS-27-275, and mixtures thereof.

63. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a neurological disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 1.

64. A method of claim 63, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

65. A method of claim 63, wherein the compound is selected from the group consisting of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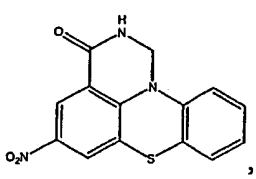
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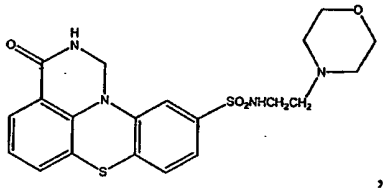

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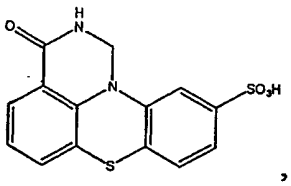

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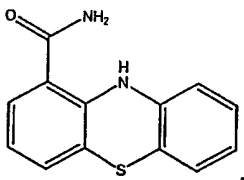

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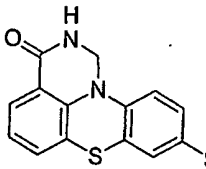

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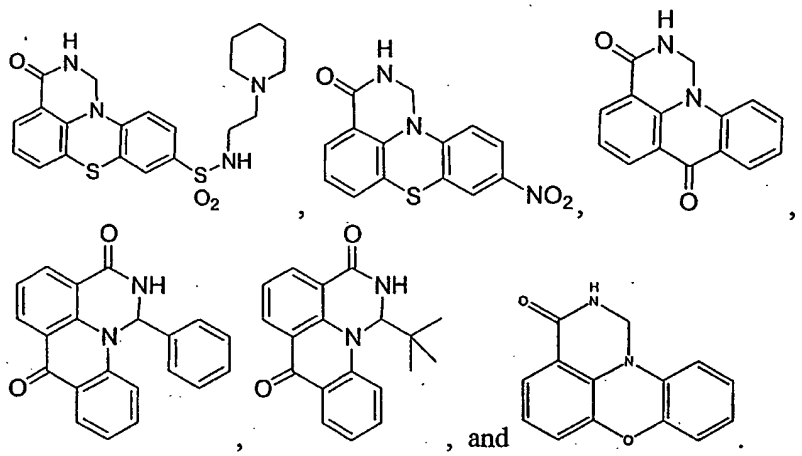
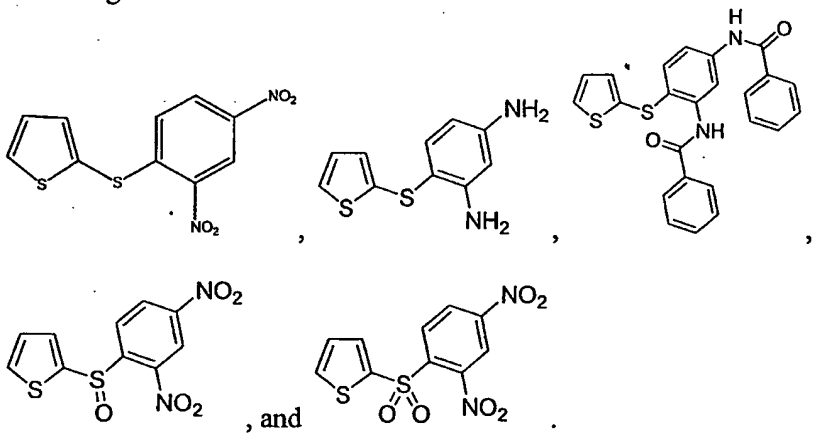


66. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a neurological disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 4.

67. A method of claim 66, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

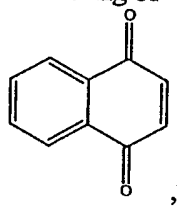
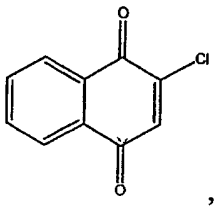
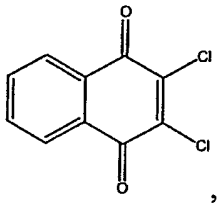
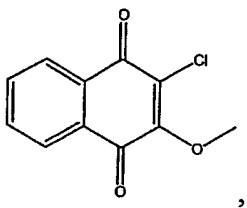
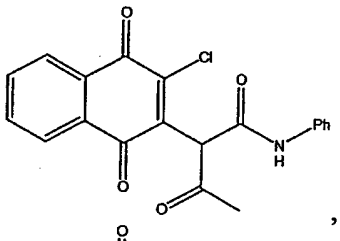
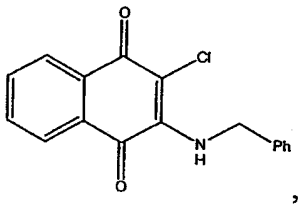
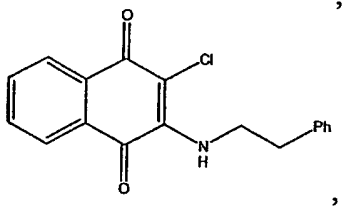
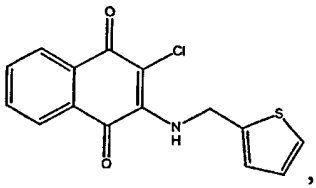
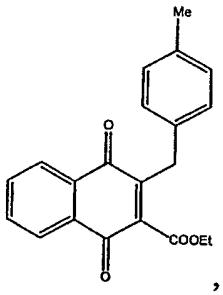
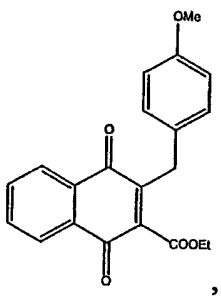
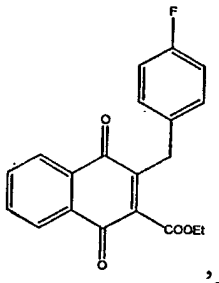
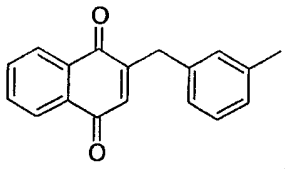
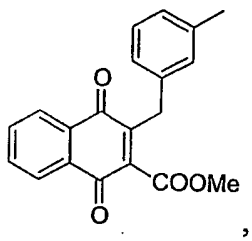
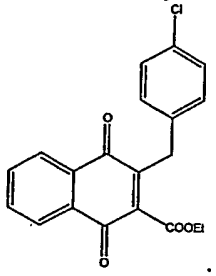
68. A method of claim 66, wherein the compound is selected from the group consisting of



69. A method comprising administering an effective amount of a NAD+-dependent deacetylase inhibitor to treat a neurological disorder wherein said NAD+-dependent deacetylase inhibitor is a compound of claim 7.

70. A method of claim 69, wherein R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11 and R12 can be optionally substituted H, F, Cl, Br, I, amino, hydroxy, -N-N, -CO-N-N, halogen-substituted amino, -O-alkyl, -O-aryl, or an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, -COR10, where R10 is H, and -OH.

71. A method of claim 69, wherein the compound is selected from the group consisting of














and 

72. A method of claim 63, 66, or 69 wherein the neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, and mixtures thereof.

73. A method of claim 72 wherein the cardiovascular disorder comprises peripheral neuropathy caused by physical injury or disease state.

74. A method of claim 72 wherein the cardiovascular disorder comprises traumatic brain injury.